(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 3 July 2003 (03.07.2003)

PCT

(10) International Publication Number WO 03/054162 A2

(51) International Patent Classification7:

C12N

- (21) International Application Number: PCT/US02/41014
- (22) International Filing Date:

19 December 2002 (19.12.2002)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10/029,397 201

20 December 2001 (20.12.2001) US

- (71) Applicant (for all designated States except US): AMBION, INC. [US/US]; 2130 Woodward St., Suite 200, Austin, TX 78744-1832 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): MURPHY, George, L. [US/US]; 8825 Escabosa Dr., Austin, TX 78748 (US). WHITLEY, J., Penn [US/US]; 5203 Avenue F, Austin, TX 78751 (US).
- (74) Agent: SHISHIMA, Gina, N.; Fulbright & Jaworski L.L.P., Suite 2400, 600 Congress Avenue, Austin, TX 78701 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

2 A2

(54) Title: METHOD AND SYSTEM FOR DEPLETING rRNA POPULATIONS

(57) Abstract: The present invention concerns a system for isolating, depleting, or separating a targeted nucleic acid, such as rRNA, from a sample comprising targeted and nontargeted nucleic acids. It effects a way of enriching for nontargeted nucleic acids, such as mRNAs. The invention further concerns methods of implementing the system and kits for implementing the system, which involves at least one bridging nucleic acid comprising 1) a targeting region complementary to a region on the targeted nucleic acid and 2) a bridging region complementary to the capture region of a capture nucleic acid that comprises a nonreactant structure. The nonreactant structure can be used to isolate the hybridizing molecules after incubation under conditions that allows hybridization.

DESCRIPTION

1

METHOD AND SYSTEM FOR DEPLETING rRNA POPULATIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

5

10

15

20

25

30

The present invention relates generally to the fields of molecular biology and microbial pathogenesis. More particularly, it concerns methods, compositions, and kits for isolating, depleting, separating a targeted nucleic acid population from other nucleic acid populations as a means for enriching those other nucleic acid population(s). More particularly, it concerns methods, compositions, and kits for enriching mRNA populations by depleting eukaryotic and/or prokaryotic rRNA from a sample using engineered bridging and capture nucleic acid molecules.

2. Description of Related Art

The ongoing efforts in microbial genome sequencing will enable unprecedented advances in our understanding of microbes and host-microbe interactions. Dozens of prokaryotic genomes, including those of numerous human pathogens, have been completely sequenced, and many others are in progress. Consequently, a renewal of focus and energy has emerged in the fields of microbial evolution, microbial pathogenesis, and infectious diseases. The potential impact of genomics on these disciplines is the subject of several recent reviews (Cummings et al., 2000; Cornelis et al., 2001; Fox et al., 2001; Current Opinion in Microbiology). For host-microbe interactions, the ability to measure the expression of every single gene in a microorganism will make possible studies of such complex interactions as the global regulation of virulence factors and the mechanisms of response to host cells and their microenvironment. Scientists will also be able to evaluate the complete repertoire of host cell gene expression in response to the pathogen. Undoubtedly, novel interactions and responses between microbes and their hosts will be discovered, leading to a more complete picture of infectious diseases and how to control them.

In the past decade, researchers studying bacteria developed several novel approaches to evaluate global gene transcription in response to environmental stimuli, including host-microbe interactions. Prior to the era of genome sequencing, Chuang et al. (Chuang et al., 1993) used an ordered set of E. coli lambda library clones to evaluate global transcription responses of E. coli. Other groups employed subtractive hybridization and differential screening to evaluate induction

of gene expression in Mycobacterium avium after phagocytosis by macrophages (Plum et al., 1994) or in *Pyrococcus* grown under specific environmental conditions (Robinson et al., 1994). Researchers further developed this approach with an elegant procedure for the selective capture of transcribed sequences (SCOTS) (Graham et al., 1999). At the same time, many scientists bypassed library construction altogether and used using differential display (Liang et al., 1995) to discover genes that are transcribed differently under various growth conditions. Although useful in certain circumstances, differential display is frequently a hit-or-miss prospect and gives no information on global transcription. More recently, serial analysis of gene expression (SAGE) (Velculescu et al., 1995) emerged as a method for analyzing the complete transcriptome of a cell. SAGE, like differential display, can be useful but requires large amounts of nucleic acid sequencing. Not unexpectedly, for organisms whose genomes have been sequenced, array analysis is emerging as the method of choice for global gene expression studies with bacteria. Macroarrays (filter-based arrays) and microarrays (slide-based arrays) of complete genomes have made possible the simultaneous expression analysis of thousands of genes. The advent of microarray technology has already enabled analyses of the host response to interactions with pathogenic organisms (Cummings et al., 2000). Similarly, microarray analysis and other methods have been used to evaluate gene expression in bacteria grown under different environmental conditions in vitro.

5

10

15

20

25

30

The application of array analysis to gene expression profiling in prokaryotes was an immediate outgrowth of similar studies with eukaryotic organisms, occurring only within the past two to three years. Infectious disease researchers have already begun applying microarray analysis to the study of complex host-microbe interactions. To date, such analyses of host-microbe interactions have been limited to the evaluation of host cell responses to bacteria or viruses. Bordetella pertussis, Listeria monocytogenes, Neisseria meningitidis, Pseudomonas aeruginosa, Legionella pneumophila, Salmonella dublin, and Staphylococcus aureus are among the bacterial pathogens whose effects on host cell gene expression have been evaluated with microarrays. Array analyses of eukaryotic host cell transcription are feasible because of the ability to isolate polyadenylated mRNAs from eukaryotic cells and to specifically label mRNAs by oligo dT-primed cDNA synthesis.

Although it has been alluded to in the literature (Cummings et al., 2000; Rappuoli, 2000), complete genome array expression analyses of bacteria in response to interactions with host cells

have not been widely published, if at all. Studies that examine the global bacterial gene response in the presence of host cells will require the development of tools to enable the efficient isolation, enrichment, and labeling of bacterial mRNAs (Cummings et al., 2000; Graham et al., 1999; Gingeras et al., 2000; Graham et al., 2001).

However, technical limitations of current methods available for purification and evaluation of bacterial mRNAs preclude these types of whole genome analysis. To realize the full potential of the genomics revolution, methods for purifying mRNAs from total bacterial RNA populations and particularly from mixtures of host cell and bacterial RNA need to be developed.

5

10

15

20

25

30

Isolating sufficient quantities of high quality bacterial mRNA is perhaps the most demanding technical requirement impeding analyses of bacterial gene expression in the presence of host cells. A small percentage of bacterial mRNAs may be A-tailed, but these are targeted for degradation and tend to be unstable. As a result, the commonly used method for mRNA purification with eukaryotic cells, oligo-dT capture, is ineffective.

Only a few studies have described methods for enriching or purifying bacterial mRNAs. Several groups (Plum et al., 1994; Robinson et al., 1994; Su et al., 1998) have used rRNA subtraction to enrich for bacterial mRNAs. These procedures involved hybridization of rRNAs to biotinylated plasmid containing rRNA genes or to biotinylated antisense rRNAs followed by streptavidin capture and removal. This yields some benefits, but it requires fairly large amounts of plasmids or antisense RNA. Biotinylation of large amounts of DNA or RNA is often tricky and can be prohibitively expensive if biotin-modified nucleotides are incorporated during antisense RNA synthesis. In general, these methods have not seen widespread use. As mentioned above, Graham and Clarke-Curtiss (Graham et al., 1999) went further in enriching for mycobacterial mRNAs with SCOTS. The SCOTS procedure is effective for detecting genes specifically expressed in the presence of host cells but is hampered by being a multi-step procedure that requires production of normalized double-stranded cDNA, PCR, differential hybridization, and cDNA capture. In addition to these methods, researchers have developed methods to polyadenylate bacterial mRNAs, thereby allowing for their purification by oligo dTcapture. Amara and Vijaya (Amara et al., 1997) demonstrated that mRNAs in purified polysomes can be specifically polyadenylated and purified by oligo-dT capture. Wendisch et al. (Wendisch et al., 2001) showed that the same process can be carried out with crude cell extracts.

WO 03/054162 PCT/US02/41014 4

Several shortcomings are associated with the polyadenylation approach. Different mRNAs may be polyadenylated to different extents or not at all depending on the structure of their 5' and 3' ends (Feng et al., 2000). Polyadenylation in a cell lysate, followed by purification of RNA, will require inactivation of cellular RNAses so that transcripts are not degraded during the polyadenylation reaction. Optimizing the reaction to work reproducibly in many different bacterial cell lysates would likely be very difficult. Despite many worthy attempts, simple and universal procedures for bacterial mRNA enrichment, especially in the presence of host cell RNA, remain elusive. Thus, there remains a continued need for improvements in mRNA enrichment and/or the depletion of other RNA populations.

5

10

15

20

25

30

SUMMARY OF THE INVENTION

The present invention involves a system that allows for the isolation, separation, and depletion of a population of nucleic acid molecules. The system involves components that may be used to implement methods for isolating, separating, or depleting a targeted nucleic acid. Such components may also be included in kits of the invention.

In embodiments of the invention, a population of nucleic acids may be targeted for isolation, separation, or depletion. Such a nucleic acid is referred to as "targeted nucleic acid" or "targeted nucleic acid molecule." Alternatively, it may be referred to as a "nucleic acid target." In particular embodiments of the invention, the targeted nucleic acid is rRNA. In alternative embodiments, the targeted nucleic acid is mRNA, tRNA, or DNA including, cDNA and genomic DNA. The targeted nucleic acid may be in a sample, which is a composition that is suspected of containing the targeted nucleic acid. In some embodiments, the sample is obtained from or includes prokaryotes or eukaryotes or both. The sample may be cells, tissues, organs, and lysates, fractionations, or portions thereof. Furthermore, the targeted nucleic acid is targeted via a "targeting region" in the targeted nucleic acid. A "targeted region" refers to a region of the targeted nucleic acid that is complementary with the targeting region of a bridging nucleic acid and that allows the targeted nucleic acid to be separated from other non-targeted nucleic acid populations.

In embodiments in which the targeted nucleic acid is rRNA, the rRNA may be the 5S, 16S, or 23S rRNA from prokaryotes, though it may be any rRNA species from a prokaryotes. It is specifically contemplated that nucleic acids may be targeted in Gram positive bacteria and Gram negative bacteria. In further embodiments, the targeted rRNA is 5.8S, 17S or 18S, or 28S

5

rRNA (referred to as "types of rRNA") from a eukaryote. It is further contemplated that tRNA may be a targeted nucleic acid population either by itself or in combination with any of the targeted nucleic acids described herein. A non-limiting list of targeted rRNAs from various organisms is provided in a later section and is contemplated to be part of the invention.

5

10

15

20

25

30

In embodiments of the invention, the system involves a bridging nucleic acid, a capture nucleic acid, and a targeted nucleic acid, as shown, for example, in FIG. 1. While in many embodiments of the invention it is contemplated that the bridging nucleic acid and the capture nucleic acid are oligonucleotides, it is specifically contemplated that they may be Thus, any embodiment involving an oligonucleotide may be polynucleotides as well. implemented with a polynucleotide. Bridging nucleic acids, capture nucleic acids, and targeted nucleic acids of the invention may include, be at least or be at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 or more residues in length.

Furthermore, a "bridging nucleic acid" is a nucleic acid molecule that comprises a bridging region and a targeting region, while a "capture nucleic acid" is a nucleic acid molecule that comprises a capture region. It is contemplated that bridging, targeting, and capture regions of the invention may be, be at least or be at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830,

10

15

20

25

30

6

840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 residues in length.

A "bridging nucleic acid" refers to a molecule that includes nucleic acid residues or analogs and that includes at least one targeting region and at least one bridging region. A "targeting region" refers to a region of the molecule that is involved in targeting a particular nucleic acid or nucleic acid population and is thus complementary to all or part of the sequence of the targeted nucleic acid. It is further contemplated that more than one targeting region may be included in a bridging nucleic acid. The bridging nucleic acid may include or have up to 2, 3, 4, 5, 6, 7, 8, 9, 10, or more targeting regions. When there are multiple targeting regions, it is contemplated that the regions may be complementary to different, nonoverlapping sequences from the same targeted nucleic acid or they may be complementary to similar or overlapping sequences from the same targeted nucleic acid, or they may be complementary to sequences in different targeted nucleic acids. While mRNA may be targeted, it is specifically contemplated that mRNA is not targeted and thus the targeting region does not have a stretch of polypyrimidine residues, such as poly-T or poly-U to hybridize to the poly-A tail of eukaryotic mRNA. Also considered part of the invention is using single or multiple bridging nucleic acids to deplete an rRNA population. In some embodiments, a single bridging nucleic acid may contain one or more targeting regions that are complementary to different types of rRNA ("types" refer to sizes based on intact lengths). Thus, in some embodiments, the largest type of rRNA may be targeted ("largest" refers to longest nucleic acid molecule when intact, even though molecules that are no longer intact may also be targeted if they retain the sequence that is complementary to all or part of a targeting region). In still further embodiments, the second largest rRNA or the first and second largest rRNA types may be targeted by a single bridging nucleic acid with targeting regions to each or to more than one nucleic acid, each with a targeting region to a different type of rRNA. In still further embodiments, a bridging nucleic acid has a targeting region complementary to one or more of the following prokaryotic and eukaryotic rRNA types: 5S, 16S, 23S, 5.8S, 17S, 18S, and/or 28S. A bridging nucleic acid may target 1, 2, 3, 4, 5, 6, 7, or more types of rRNA, as well as any and all tRNA types, both eukaryotic and prokaryotic.

A "bridging region" in a bridging nucleic acid refers to a region that mediates an interaction with a capture nucleic acid. In further embodiments, the bridging region is a

WO 03/054162 PCT/US02/41014 7

polypurine or polypyrimidine stretch of residues. A bridging region can include a stretch of adenine or guanine residues or cytosine, uracil, or thymidine residues. In some embodiments, it is contemplated that more than one bridging region is included in a bridging nucleic acid, such as 2, 3, 4, 5, or more bridging regions.

5

10

15

20

25

30

A "capture nucleic acid" refers to a molecule that includes nucleotides or nucleotide analogs, a capture region, and a nonreacting structure. A "capture region" refers to a region that interacts with the bridging region of a bridging nucleic acid. In embodiments of the invention, the bridging region and the capture region are complementary to each other and hybridize to one another under conditions that allow for hybridization of complementary regions. In some embodiments of the invention, the capture region and bridging region are a stretch of complementary repeated nucleotides (complementary homopolymeric regions). For example, they may be homopolymeric A, T, G, C, or U. In other embodiments of the invention, however, the bridging and capture regions are any sequence, so long as they are complementary. In some embodiments of the invention, the capture region has a sequence that includes at least 5, 6, 7, 8, 9, 10 or more contiguous nucleotides of SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NOs: 87-92"). In some embodiments, the capture regions comprises any of of SEQ ID NOs 87-92.

There may be more than one nonreacting structure attached, covalently or noncovalently, to a capture nucleic acid. There may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nonreacting structures as part of a capture nucleic acid.

A capture nucleic acid also includes a "nonreacting structure," which refers to a compound that does not chemically react with a nucleic acid. In some embodiments, a nonreacting structure is a magnetic bead or rod, which allows the capture nucleic acid, a bridging nucleic acid and a target nucleic acid to be isolated from a sample with a magnetic field, such as a magnetic stand. In still further embodiments, the nonreacting structure is a bead or other structure that can be physically captured, such as by using a basket, filter, or by centrifugation. It is contemplated that a bead may include plastic, glass, teflon, silica, a magnet or be magnetizeable, a metal such as a ferrous metal or gold, carbon, cellulose, latex, polystyrene, and other synthetic polymers, nylon, cellulose, nitrocellulose, polymethacrylate, polyvinylchloride, styrene-divinylbenzene, or any chemically-modified plastic or any other nonreacting structure. In still further embodiments, the nonreacting structure is biotin or iminobiotin. Biotin or

iminobiotin binds to avidin or streptavidin, which can be used to isolate the capture nucleic acid and any hybridizing molecules. Furthermore, in some embodiments of the invention, the nonreacting structure is cellulose or an analog thereof.

5

10

15

20

25

30

8

It is contemplated that the location of the targeting and bridging regions in the bridging nucleic acid may be at a variety of positions. The location of targeted regions in a targeted nucleic acid or a capture region in a capture nucleic acid may also vary. The location of any of these regions or nonreacting structure may be or be within 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 12, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 or more nucleotides from the 3' and/or 5' end of the relevant nucleic acid ("relevant nucleic acid" refers to the nucleic acid in which the region is located). Moreover, it is contemplated that a region, such as a bridging, capture, targeted, or targeting region—as well as a nonreacting structure—may be at or within 100-5000 residues, 150-4000 residues, 200-3000 residues, 250-2000 residues, 300-1500 residues, 350-1000 residues, 400-900 residues, 450-800 residues, or 500-700 residues of the 5' or 3' end of the relevant nucleic acid.

Furthermore, it is also contemplated that the spacing between regions may vary. Regions in the same nucleic acid or a region and a nonreacting structure may be adjacent to one another or there may be residues between them or between each of them. The number of intervening residues may be the following or may be at least or at most of the following: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590,

15

20

25

30

600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, or more nucleotides between them or each of them.

As for the location of the sequence to which the targeting region is complementary, termed "targeted region," this may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 10 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 nucleotides or more from the 3' and/or 5' end of the targeted nucleic acid. It is specifically contemplated that the targeting region hybridizes to a sequence located between 100 and 5000, 150 and 4000, 200 and 3000, 250 and 2000, and 300 and 1000 residues of the 5' and/or 3' end of the targeted nucleic acid. It is also contemplated that the targeted region is at the 3' or 5' end of the targeted nucleic acid. Alternatively, the targeted region may not be within 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 or more nucleotides from the termini of a targeted nucleic acid.

In some embodiments, the targeting region comprises or is complementary to all or 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940,

950, 960, 970, 980, 990, 1000 or more contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEO ID NO:10, SEO ID NO:11, SEO ID NO:12, SEO ID NO:13, SEO ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEO ID NO:32, SEO ID NO:33, SEO ID NO:34, SEO ID NO:35, SEO ID NO:36, SEO ID NO:37, SEO ID NO:38, SEO ID NO:39, SEO ID NO:40, SEO ID NO:41, SEO ID NO:42, SEO ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:72, or SEQ ID NO:73 (collectively referred to as "SEQ ID NOS:1-73"), as well as SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NOS:1-92"). It is specifically contemplated that targeting regions of the invention comprise, in some embodiments, at least 5 contiguous nucleotides of SEQ ID NO:1-22; it is also contemplated that targeting regions of the invention are complementary to a sequence ("sequence" in the context of complementary regions refers to a sequence of at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, or more nucleotides in length) of SEQ ID NOS:23-86, which are sequences of rRNA molecules.

10

15

20

25

30

It will be understood that any embodiment discussed with respect to nucleotides applies also when nucleotide analogs are used. It is specifically contemplated that nucleotide analogs may be employed with respect to bridging and capture nucleic acids of the invention.

It is contemplated that nucleic acids of the invention include RNA, DNA, locked nucleic acidTM (LNA), iso-bases, and/or peptide mimetics. It is contemplated that all or part of nucleic acids of the invention may include such nucleic acid components.

The present invention further concerns methods of isolating and/or depleting nucleic acids from a sample. In some embodiments, methods include a) incubating a sample with a first

5

10

15

20

25

30

bridging nucleic acid comprising (1) at least one bridging region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the first targeting region and the targeted nucleic acid; b) incubating the first bridging nucleic acid with a capture nucleic acid comprising a nonreacting structure and a capture region comprising at least 5 nucleic acid residues, under conditions that allowing hybridization between the first bridging region and the capture region. In additional embodiments, one or more other steps may be included in combination with the method discussed above. Other steps involve isolating the targeted nucleic acid from the remainder of the sample; discarding the portion of the sample that hybridizes directly or indirectly to the capture nucleic acid (indirect hybridization refers to specific association of compounds that occurs through hybridization with a mediating compound, for example, indirect hybridization of a capture nucleic acid and a targeted nucleic acid via hybridization to a bridging nucleic acid); incubating the sample with additional bridging nucleic acids, under conditions allowing hybridization between the targeting region of the additional bridging nucleic acid and the targeted nucleic acid; implementing the method with respect to other targeted nucleic acids; washing the capture nucleic acid after incubation with the sample and the bridging nucleic acid, incubating the capture nucleic acid, bridging nucleic acid, and sample with elution buffer after isolating the targeted nucleic acid from the rest of the sample; eluting the targeted nucleic acid from the nonreactant structure; using the capture nucleic acid in a subsequent method involving a new sample; discarding the targeted nucleic acid after separating it from the sample; performing hybridizations between the bridging nucleic acid and the sample and the capture nucleic acid and the sample at the same temperatures or at different temperatures; performing the above hybridization steps at the same time, sequentially (one after the other or the other after the one); exposing the sample to a magnetic field or magnet, particularly when a magnetic bead or other object comprises all or part of the non-reacting structure of the capture nucleic acid; and incubating the sample with streptavidin or avidin, particularly if biotin or iminobiotin is used as a non-reacting structure.

In some embodiments of the invention, the sample, a bridging nucleic acid and/or a capture nucleic acid are incubated in a buffer, which, in some embodiments, includes TEAC or TMAC.

In methods of the invention involving more than one bridging nucleic acid, it is contemplated that the targeting region of the first bridging nucleic acid may be complementary to

12

a different sequence of a different targeted nucleic acid than a targeting region of another bridging nucleic acid. Alternatively, different bridging nucleic acids may have targeting regions that are complementary to the same targeted nucleic acid. In the latter case, it is further contemplated that the targeting regions be complementary to sequences that overlap one another or ma be complementary to sequences in non-overlapping locations.

5

10

15

20

25

30

In cases in which targeting regions are complementary to different targeted nucleic acids, embodiments may involve targeting the largest rRNA molecule in a sample with one bridging nucleic acid and the second largest rRNA molecule in a sample with another bridging nucleic acid. In still further embodiments, another or third bridging nucleic acid will target the third largest rRNA molecule in a sample, while another or a fourth bridging nucleic acid will target the fourth largest rRNA molecule in a sample.

In another embodiment of the invention, there is a method for depleting rRNA from a sample comprising incubating the sample with (1) at least a first bridging oligonucleotide comprising a bridging region comprising a polypurine region of at least 5 residues in length and a targeting region comprising at least 5 contiguous residues complementary to an rRNA molecule in the sample and (2) a capture oligonucleotide comprising a magnetic bead and a capture region comprising a polypyrimidine region of at least 5 residues in length, under conditions allowing hybridization between the bridging oligonucleotide and the capture oligonucleotide and between the bridging oligonucleotide and the rRNA; b) incubating the sample with a magnetic bead; and c) isolating the magnetic bead. In still further embodiments, the first bridging oligonucleotide comprises a targeting region complementary to prokaryotic 23S rRNA. In still further embodiments, there is a second bridging oligonucleotide with a targeting region complementary to a different region of a prokaryotic 23S RNA than the first bridging In even further embodiments, there is a third and fourth bridging oligonucleotide. oligonucleotide each with a targeting region complementary to different sequences of a prokaryotic 16S rRNA.

As discussed earlier, a sample may be depleted or isolated as a way of enriching for the nontargeted nucleic acid, such as mRNA. In further embodiments of the invention, enriched mRNA can be used to prepare cDNA according to methods known to those of ordinary skill in the art, and as described herein. Thus, in cases in which mRNA is enriched as a result of methods of the invention, embodiments may further include discarding the portion of the sample

13

that hybridizes to the capture oligonucleotide. More specifically targeted rRNA may be discarded and the mRNA remaining in the sample may be used to produce cDNA molecules. cDNA molecules may be used in a variety of methods, including, but not limited to, library production, production of proteins, and for creating and screening arrays. Therefore, in some embodiments of the invention, cDNA made from mRNA enriched according to methods of the invention are attached to a solid support or surface so as to create a nucleic acid array. The term "nucleic acid array" refers to a plurality of target elements, wherein each target element comprising one or more nucleic acid molecules immobilized on one or more solid surfaces at discrete locations to which sample nucleic acids can by hybridized. The nonreacting solid surface or support may be any of a number of materials, including plastic, glass, or nylon. In some embodiments, the solid support is a plate. The plate may have wells that contain the target elements. Plates may have 2, 3, 4, 5, 6, 7, 8, 9, 10 or more wells ("multi-well"), and up to at least 96 or 192 wells. In some embodiments of the invention, the sample nucleic acids comprise cDNAs made by depleting a sample of rRNA, according to methods of the invention. Those embodiments may further involve contacting a nucleic acid array with the cDNA. Alternatively, cDNA made according to the invention may be used as target elements on an array. In any of these embodiments of the invention, it is specifically contemplated that enriched mRNA may be amplified into RNA or DNA by techniques known to those of skill in the art and then used in methods of the invention, such as to probe or screen an array.

5

10

15

20

25

30

The present invention also concerns kits that include compositions of the invention to implement the methods discussed herein. These kits can be used for the depletion, isolation, or purification of nucleic acids. Kits contain these compositions in a suitable container means.

In some embodiments, a kit includes 1) at least one capture oligonucleotide comprising a capture region and a magnetic bead; and 2) at least a first bridging oligonucleotide comprising i) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and ii) at least one targeting region comprising 10 contiguous nucleic acids complementary to an rRNA.

In additional embodiments, there is a second bridging oligonucleotide comprising i) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and ii) at least one targeting region comprising 10 contiguous nucleic acids complementary to an rRNA. In some kits, the targeting region of the second bridging

14

oligonucleotide is complementary to the same rRNA as the targeting region of the first bridging oligonucleotide, while in other embodiments, these are complementary to different rRNAs. Further embodiments involve kits in which the targeting region of the first bridging oligonucleotide is complementary to the largest rRNA of a prokaryote or eukaryote. In other embodiments, the second bridging oligonucleotide has a targeting region that is complementary to either the largest rRNA of a prokaryote or eukaryote or the second largest rRNA of a prokaryote or eukaryote. It is specifically contemplated that kits may include one or more bridging oligonucleotides targeting prokaryotic rRNA (16S, 23S, or both) and one or more bridging oligonucleotides targeting eukaryotic rRNA (18S, 28S, or both); thus, a kit may be used for depleting both eukaryotic and prokaryotic rRNA, in some embodiments.

5

10

15

20

25

Kits may also include a third, fourth, fifth, sixth, seventh, eighth, ninth, tenth or more bridging oligonucleotides with targeting region complementary to the same or different rRNAs as the targeting regions of the first and second bridging oligonucleotides. It is contemplated that the targeting regions of the bridging oligonucleotides in kits of the invention may be complementary to prokaryote 16S rRNA, prokaryote 23S rRNA, prokaryote 5S rRNA, eukaryote 17S or 18S rRNA, eukaryote 28SrRNA, and/or eukaryote 5.8S rRNA. It is further contemplated that targeting regions of bridging oligonucleotides in kits may have all or part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22 (collectively referred to as "SEO ID NOS:1-22"). Alternatively, kits may include targeting regions as discussed above with respect to SEO ID NOS:23-86, i.e. targeting regions complementary to a sequence from SEQ ID NOS:23-86. Kits of the invention may also include one or more of the following: binding buffer with TMAC. binding buffer with TEAC, magnetic stand, wash solution, nuclease-free water; RNAse inhibitors, glycogen, control RNA, sodium acetate, ammonium acetate, streptavidin beads, avidin beads, magnetic beads, beads of any nonreacting structure--including those discussed above--capture basket; capture filters, RNA markers, nuclease-free containers such as tubes and tips, and any other composition described herein.

15

It is contemplated that kits of the invention may be used to implement methods of the invention, that methods of the invention may be implemented with compositions of the invention, and that kits may include any composition of the invention.

It is further contemplated that kits, methods, and compositions of the invention may effect a depletion of a targeted nucleic acid in a sample by reducing its amount in the sample by at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or more percent.

5

10

15

20

25

Kits of the invention also include materials for creating a nucleic acid array and probing a nucleic acid array. Any of the kits discussed above may also include a solid support for preparing a nucleic acid array.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." When the term "at least" is used in the context of bridging, targeting, or capture regions, as well as for capture and bridging oligonucleotides, it is contemplated that there is an upper limit of 20 for practical purposes, even though more such regions or oligonucleotides could be implemented with the invention. Furthermore, it should be understood that a number (cardinal or ordinal) used in the context of compositions of the invention refers to a "kind" of that composition; thus, "a first oligonucleotide" in the context of a "second oligonucleotide" refers to "one of that kind of oligonucleotide," and not one single oligonucleotide molecule.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

10

15

20

25

30

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Depiction of molecules in system. A bridging oligonucleotide is shown with a targeting region and a bridging region. The targeting region is complementary to a targeted region in the targeted nucleic acid, which is an rRNA molecule. The bridging region is complementary to the capture region in the capture oligonucleotide, which is attached, by way of example, to a magnetic bead as a nonreacting structure.

FIG. 2A-1 to A-14 and FIG. 2B-1 to B-27. Sequence comparison of different rRNAs from different bacteria to E. coli rRNA with MegAlign sequence analysis software version 4.05 from DNA Star, Incorporated. A. The 5' end of the sequence is shown on the first page of the figure in FIG. 2A-1 and continues until the last page of the figure, FIG. 2A-14, in which the 3' end of the same sequence is shown. Shown is a sequence comparison of 16S rRNA of listed prokaryotic organisms to 16S rRNA from E. coli (SEQ ID NO:34). The sequences are the 16S rRNA from the following organisms: B. subtilis (SEQ ID NO:23); B. anthracis (SEQ ID NO. 24); E. faecalis (SEQ ID NO. 25); L. lactis (SEQ ID NO. 26); L. monocyt (SEQ ID NO. 27); S. aureus (SEQ ID NO. 28); S. mutans (SEQ ID NO. 29); S. pneumon (SEQ ID NO. 30); S. pyogenes (SEQ ID NO. 31); M. avian (SEQ ID NO. 32); M. tuberculosis (SEQ ID NO. 33); K. pneumoniae (SEQ ID NO. 35); A. actino (SEQ ID NO. 36); H. influenzae (SEQ ID NO. 37); E. bronchiseptica (SEQ ID NO. 38); B. parapertussis (SEQ ID NO. 39); B. pertussis (SEQ ID NO. 40); B. cepacia (SEQ ID NO. 41); B. mallei (SEQ ID NO. 42); B. pseudomallei (SEQ ID NO. 43); N. gonorrhoeae (SEQ ID NO. 44); N. mening (SEQ ID NO. 45); P. aeruginosa (SEQ ID NO. 46); V. cholerae (SEQ ID NO. 47); and Y. enterocolitica (SEQ ID NO. 48). B. The 5' end of the sequence is shown on the first page of the figure in FIG. 2B-1 and continues until the last page of the figure, FIG. 2B-27, in which the 3' end of the same sequence is shown. Shown is a sequence comparison of 23S rRNA of listed prokaryotic organisms to 23S rRNA from E. coli (SEQ ID NO:60). The sequences are the 23S rRNA from the following organisms: B. subtilis (SEQ ID NO:49); B. anthracis (SEQ ID NO. 50); E. facaelis (SEQ ID NO. 51); L. lactis (SEQ ID

NO. 52); L. monocytogenes (SEQ ID NO. 53); S. aureus (SEQ ID NO. 54); S. mutans (SEQ ID NO. 55); S. pneumoniae (SEQ ID NO. 56); S. pyogenes (SEQ ID NO. 57); M. avium (SEQ ID NO. 58); M. tuberculosis (SEQ ID NO. 59); K. pneumoniae (SEQ ID NO. 61); H. influenzae (SEQ ID NO. 62); B. bronchiseptica (SEQ ID NO. 63); B. parapertussis (SEQ ID NO. 64); B. pertussis (SEQ ID NO. 65); B. cepacia (SEQ ID NO. 66); E. mallei (SEQ ID NO. 67); E. pseudomallei (SEQ ID NO. 68); N. gonorrhoeae (SEQ ID NO. 69); N. eminigititdis (SEQ ID NO. 70); P. aeruginosa (SEQ ID NO. 71); V. cholerae (SEQ ID NO. 72); Y. enterocolitica (SEQ ID NO. 73).

5

15

- FIG. 3. Electropherograms of RNA from a control reaction. *E. coli* total RNA was purified with RNAwiz^M (Ambion) and carried through the rRNA depletion procedure as described in Example 2, except that bridging nucleic acids were left out of the reaction. A sample of the RNA was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
 - FIG. 4. Electropherograms of RNA from an experimental reaction after ribosomal RNA depletion. *E. coli* total RNA was purified with RNAwiz[™] (Ambion) and carried through the rRNA depletion procedure as described in Example 2. A sample of the RNA was analyzed as described in the legend to FIG. 3.
- Electropherograms of RNA from experiments. FIG. 5A-B. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in 20 Example 5, but with no bridging oligonucleotides. The sample contains E. coli and rat liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. 25 Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 5 with bridging oligonucleotides. The sample is depleted of E. coli 16S and 23S rRNA and rat liver 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 30 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

10

15

20

25

30

- FIG. 6A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 6, but with no bridging oligonucleotides. The sample contains human liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 6 with bridging oligonucleotides. The sample is depleted of human 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
- FIG. 7A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 7, but with no bridging oligonucleotides. The sample contains rat liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 6 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
- FIG. 8A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 6, but with no bridging oligonucleotides. The sample contains mouse liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 8 with bridging oligonucleotides. The sample is depleted of mouse 18S and 28S

The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper rRNA. Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

- 5 FIG. 9A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 11 with no bridging oligonucleotides. The sample contains human total RNA (50 µg) and E. coli total RNA (500 ng). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software 10 (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 11 with bridging oligonucleotides. The sample is depleted of human 18S and 28S rRNA, but E. coli total RNA remains in the The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). 15 electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
- FIG. 10A-C. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction with no bridging oligonucleotides 20 performed as described in Example 12. The sample contains rat liver total RNA (25 µg) and E. coli total RNA (2 μg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an 25 experimental reaction performed as described in Example 12 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA, but E. coli total RNA remains in the sample. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). C. Agilent 30

10

15

20

25

30

WO 03/054162 PCT/US02/41014 20

2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 12 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA and E. coli 16S and 23S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 11A-B. E. coli gene arrays probed with cDNA from experiments. Experimental reactions were performed as described in Example 13. RNA samples were used to generate radiolabeled cDNA for use as probes with replicate portions of Sigma-Genosys PanoramaTM E. coli gene arrays. A. E. coli gene array probed with a sample from a control reaction. The sample contains human total RNA (25 µg) and E. coli total RNA (2 µg). B. E. coli gene array probed with an RNA sample that was depleted of human 18S and 28S rRNA and E. coli 16S and 23S rRNA.

FIG. 12A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains Campylobacter fetus total RNA (10 μg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 µg of Campylobacter fetus total RNA that employed the bridging oligonucleotides d16S-807, d16S-1092, d23S-479CH, and d23S-2511. The sample is depleted of 16S rRNA, 23S rRNA fragment (1260 nt), and 23S rRNA fragment (1667 nt). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer The electropherogram shown was generated with Agilent 2100 (Agilent Technologies). Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 13A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains Rhodobacter sphaeroides total RNA (10 µg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper

21

Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). **B.** Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 μg of *Rhodobacter sphaeroides* total RNA that employed the bridging oligonucleotides d16S-537 (16 pmol), d16S-1114R(16 pmol), d23S-479CH (16 pmol), d23S-1954 (16 pmol), and d23S-2511 (16 pmol). The sample is depleted of the 16S rRNA and the 23S rRNA fragment that co-migrates with the 16S rRNA. The sample is also depleted of the 23S rRNA fragment (1260 nt). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 14A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains *Anabaena sp.* total RNA (10 μg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 μg of *Anabaena sp.* total RNA that employed the bridging oligonucleotides d16S-364 (12.5 pmol), d16S-1087CY (12.5 pmol), d23S-485 (20 pmol), and d23S-1954 (35 pmol). The sample is depleted of 16S rRNA and the 23S rRNA fragments at 520 nt, 2090 nt, and 2470 nt. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

25

30

5

10

15

20

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention concerns a system for isolating, depleting, or identifying specific, targeted nucleic acid populations, such as rRNA in a sample, in some cases for the purpose of enriching for other nucleic acid populations. The targeted nucleic acid, components of the system, and the methods for implementing the system, as well as variations thereof, are provided below.

I. Targeted Nucleic Acid

5

10

15

20

25

30

The present invention concerns targeting a particular nucleic acid population (i.e., mRNA, rRNA, tRNA, genomic DNA) or targeting types of a nucleic acid population, such as individual tRNAs, rRNAs (5S, 16S, or 23S rRNA from prokaryotes; 5.8S, 17S or 18S, or 28S from eukaryotes), or specific mRNAs. A nucleic acid is targeted by using a bridging nucleic acid that has a targeting region—a region complementary to all or part of the targeted nucleic acid.

In some embodiments, the invention is specifically concerned with depleting or isolating rRNA from other nucleic acids ("non-targeted nucleic acids" or "enriched population"). The 5S, 16S, and/or 23S rRNA from a prokaryote may be the targeted nucleic acid. Also, the 5.8S, 17S (observed in yeast) or 18S, and/or 28S from a eukaryote may be the targeted nucleic acid. Alternatively, rRNAs from both prokaryotes and eukaryotes may be targeted, such as with a sample that has eukaryotic host cells infected with a prokaryotic organism. The sequences for ribosomal RNAs are well known to those or ordinary skill in the art and can be readily found in sequence databases such as GenBank (www.ncbi.nlm.nih.gov/) or are published. Nucleic acids may be targeted by targeting regions that are complementary to all or part of the targeted nucleic acid. Targeted nucleic acids may be, be at least, or be at most 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, or more nucleotides in length. Furthermore, any region of at least five contiguous nucleotides in the targeted nucleic acid may be used as the targeted region—that is, the region that is complementary to the targeting region of a bridging nucleic acid. Also, there may be more than one targeted region in a targeted nucleic acid. There may be, be at least, or be at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or

10

15

20

25

30

more targeted regions in a targeted nucleic acid. A targeted region may be a region in a targeted nucleic acid that has greater than 70%, 80%, or 90% homology with a sequence from a different targeted nucleic acid. In some embodiments, the targeted region from a targeted nucleic acid is identical to a sequence in a different targeted nucleic acid. For example, 23S rRNA of various prokaryotes may be targeted using a targeted region common to a group of organisms, such as Gram negative bacteria or a subset of such bacteria. Alternatively, a targeted region may be a sequence unique to a particular targeted nucleic acid. However, for purposes of this application, a "targeted region" is not a poly-A region, such as a poly-A tail of an eukaryotic mRNA. Additional information regarding targeted rRNAs is provided below. This information is provided as an example of targeted nucleic acids. However, it is contemplated that there may be sequence variations from individual organism to organism and these sequences provided as simply an example of one sequenced nucleic acid, even though such variations exist in nature. It is contemplated that these variations may also be targeted, and this may or may not require changes to a targeting nucleic acid or to the hybridization conditions, depending on the variation, which one of ordinary skill in the art could evaluate and determine.

A number of patents concern a targeted nucleic acid, for example, U.S. Patent Nos. 4,486,539; 4,563,419; 4,751,177; 4,868,105; 5,200,314; 5,273,882; 5,288,609; 5,457,025; 5,500,356; 5,589,335; 5,702,896; 5,714,324; 5,723,597; 5,759,777; 5,897,783; 6,013,440; 6,060,246; 6,090,548; 6,110,678; 6,203,978; 6,221,581; 6,228,580; and WO 01/32672, all of which are specifically incorporated herein by reference.

A. Prokaryotic rRNA

Prokaryotic rRNA can be a targeted nucleic acid of the invention. The following examples are provided, but the invention is not limited solely to these organisms and sequences (GenBank accession number provided and/or region within sequence that corresponds to the targeted rRNA):

1. Superkingdom Archaea (archaebacteria)

Aeropyrum pernix

16S

D83259

Aeropyrum pernix NC_000854

APErRNA05 (16S)

APErRNA03 (23S)

Methanococcus jannaschii

16S

M59126

PCT/US02/41014 24

•		us jannaschii NC_0009	109	
		A16S	157985-159459	
	MJrm		154759-157648	
_		n marismortui		
5	238	1100 1 110 110	X13738	
		n sp. NRC-1 NC_00260		
	rrs (16		1875505-1876977	
	πlA (i Thermoplasm		1877506-1880411	
10	23S	1 асіаорпішт	M32298	
10		a acidophilum NC_002		
	16S	* ### T.O_002	1475300-1475770	
2.		om <u>Eubacteria</u> (euba		
	a. Firm	icutes (Gram-positiv	e bacteria)	
15	i)	Bacillus/Clostridiu bacteria)	ım group (low G+C gram-posi	tive
		Listeria innocua Clip	11262 NC 003212	
		16S	260527-262081	
		23S	262327-265257	
20				
·		Listeria monocytogen	es strain EGD NC_003210	
		16S	237466-239020	
		23S	239265-242195	
35		D : 11 1 - 11 - 2700	20004	
25		Bacillus subtilis NC0		
		RmO 16S RmA 23S	9809-11361 11707-14634	
		KIIA 233	11707-14634	
		Bacillus anthracis		
30		16S (1508nt)	AF155950	
		23S (2922nt)	AF267877	
		Bacillus thuringiensis	;	
	٠.,	16S (1486nt)		
35		23S (2923nt)	AF267880	
		Staphylococcus aureu	s strain Mu50 NC 002758	
		16S	530479-532033	
		23S	532398-535231	
40				
		Staphylococcus aureu	=	
		SarRNA01 16		
		SarRNA02 23	3S 508166-510999	
45		Clostridium acetobuty	plicum ATCC824 NC_003030	
		16SarRNA	9710-1121 9	
		23SarRNA	11398-14303	
		Clostridium difficile		
.50		16S (1470nt)	X73450	
		103 (14/0111)	X13430	

	Clostridium perfringens		
	16S	M69264 (499-2294)	
	Musonlasma assisalisma G2'	7 1 42067	
5	Mycoplasma genitalium G3' MgrmA16S	170009-171527	
	MgrrnA23S	171730-174463	
	_	· · · · · · · · · · · · · · · · · · ·	
•	Mycoplasma pneumoniae N		
10	168	118312-119824	
10	23S	120057-122961	
	Mycoplasma pulmonis NC_	002771	
	16S	813583-815113	
	238	810563-813297	
15	Streptococcus pneumoniae R6 NC_003098		
	RRNA16S-1	15161-16674	
	RRNA23S-1	16945-19846	
20	Ct	TIOD 4 A DOOLGTO	
20	Streptococcus pneumoniae 7 SprnaA16S	15394-16806	
	SprrnaA23S	17142-20043	
	эрниа л 233	17142-20043	
	Streptococcus pyogenes AE		
25	16S	17170-18504	
	238	19037-21937	
	Streptococcus mutans		
	16S (1334nt)	X58303	
30	238	AF139599 (1940-4840)	
·	Lactococcus lactis		
	16S	X64887 (508-2055)	
	238	X64887 (2360-5257)	
35	T A T P		
	Enterococcus faecalis	Y18293	
	16S (1449nt) 23S (2912nt)	AJ295306	
	233 (2912111)	AJ295500	
40 ii)		Actinobacteria (high G+C gram-positive bacteria	
	Mycobacterium leprae strair	1 TN NC_002677	
	Rrs16S	1341144-1342692	
	Rrl23S	1342976-1346100	
45	Mycobacterium tuberculosis	CDC 1551 NC_002755	
	MtrmaA16S	1471388-1472923	
	MtrmaA23S	1473199-1476336	
	Mycobacterium avium		
50	16S (1372nt)	M61673	
	238	X74494 (295-3401)	

Corynebacterium glutamicum 16S (1479nt) Z46753 Rhodococcus equi 5 16S (1478nt) X80614 b. Spirochaetales (spirochetes) Borrelia burgdorferi AE000783 RrlB 16S 444581-446118 10 RrlB 23S 438590-441508 Treponema pallidum AE000520 TprmaA16S 230162-231656 TprmaA23S 231950-234850 15 Borrelia burgdorferi AE001147 (9459-10996) 16S **23S** AE001147 (212-3145) 20 **Thermotogales** c. Thermotoga maritima AE000512 TmrrnaA16S 188968-190526 TmrmaA23S 190766-193787 25 d. Thermus/Deinococcus group Deinococcus radiodurans R1 NC_001263 DrrmaA16S 2285518-2287019 DrrmaA23S 2245319-2246194 30 Deinococcus radiodurans AE002076 (7275-8776) 16S **23S** AE001886 (8829-10771) Chlamydiales (chlamydias) e. 35 Chlamydia trachomatis AE001273 16SrRNA1 854128-855677 23SrRNA1 855993-858862 Chlamydophila pneumoniae AR39 NC_002179 40 1069329-1070785 CprmA16S CprmA23S 1066159-1069022 Chlamydophila psittaci U68447 (1-1553) 16S 45 **23S** U68447 (1778-4721) f. Proteobacteria (purple bacteria) i) Alpha subdivision

Rickettsia conorii Malish 7 NC_003103

27

	Rrs16S 884601-886108
	Rrl23S 281797-284557
	Rickettsia prowazekii strain Madrid E AJ235269
5	Rrs16S 772263-773769
	Rrl23S 257853-260613
	Rickettsia typhi
	16S (1444nt) M20499
10	23S Y13133 (956-3716)
	Ehrlichia bovis
	16S (1488nt) U03775
15	Agrobacterium tumefaciens C58 AE007870
	16S 768991-770427
	238 765313-767565
	Brucella melitensis
20	16S AF220148 (645-2129)
	23S AF220148 (2896-30243204-5807)
	Rhizobium rhizogenes
25	16S (1369nt) D13945
ii)	Beta subdivision
	Neisseria meningitides strain MC58 AE002098
	NmrrnaA16S 60971-62514
30	NmrrnaA23S 63178-66068
	Bordetella bronchiseptica
	16S (1532nt) X57026
	23S (2865nt) X70371
35	Bordetella parapertussis
	16S (1464nt) U04949
	23S (2865nt) X68368
	Bordetella pertussis
40	16S (1464nt) U04950
	Burkholderi mallei
	16S (1488nt) AF110188
45	23S (2882nt) Y17183
7.7	Burkholderi pseudomallei
	16S (1488nt) U91839
	23S (2882nt) Y17184
50	Neisseria gonorrhoeae
	16S (1544nt) X07714
	23S (2890nt) X67293

	iii)	Gamma group	
		Buchnera sp. APS NC_002528 Rrs 16S	274065-275524
5		Rrs 165 Rrl 23S	539539-542451
3		KH 235	JJ7JJ7-J4Z4J1
	Escherichia coli K12 U0009		
		RrsH 16S	223771-225312
		RrlH 23S	225759-228662
10		Escherichia coli 0157:H7 NC	002695
		RrsH 16S	227102-228643
		RrlH 23S	229090-231992
			1: 2:0 000400
15		Salmonella enterica serovar Ty	
		16S	287479-289020
		23S	289375-292380
		Salmonella typhimurium LT2 NC_003197	
20		RrsH16S	289189-290732
		RrlH23S	291244-294336
		Yersinia pestis NC_003143	
		16S	12292-13763
25		238	14272-17178
•		Klebsiella pneumoniae	
		16S (1534nt)	X87276
20		23S (2903nt)	X87284
30		Yersinia enterocolitica	
		16S (1484nt)	Z49830
		23S (2906nt)	U77925
25		n tourt	
35		Proteus vulgaris	X07652
		16S (2067nt)	AU/032
		Shigella flexneri	
		16S (1468nt)	X80679
40		Chigalla gannai	
		Shigella sonnei 16S (1467nt)	X80726
		103 (140/111)	A00/20
		Shigella dysenterica	
45		16S (1487nt)	X96966
		Hammahilan in Arrana Da TA	2002
		Haemophilus influenzae Rd L4 HirmE16S	1511137-1512634
		HirmE23S	123801-126697
50		11IIIIE233	123001-12007/
		Pasteurella multocida	
		16S (1543nt)	M35018
		, a a (a a)	

		Actinobacillus actinomycetemc 16S (1485nt)	omitans M75037
5		Actinobacillus pleuropneumoni 168	ae D30032 (83-1625)
10		Haemophilus somnus 16S (1483nt)	M75046
		Legionella pneumophila 16S (1544nt)	M59157
15		Mannheimia haemolytica 16S (1472nt)	U57072
		Vibrio cholerae chromosomel 1 16Sa rRNA 23Sa rRNA	NC_002505 53823-55357 55784-58670
20		Vibrio parahaemolyticus 16S (1499nt)	M59161
25		Coxiella burnetii 16S (1484nt) 23S	M21291 X79704 (1620-3350)
30		Aeromonas hydrophila 16S (1538nt)	X87271
		Aeromonas salmonicida 16S (1502nt)	X60405
35		Francisella tularesis 16S (1517nt)	Z21931
		Moraxella catarrhalis 16S (1511nt)	U10876
40		Pseudomonas aeruginosa AE0 16S	722096-726631
		23S Pseudomonas putida	724103-726993
45		16S (1527nt)	D84020
	iv)	Delta/Epsilon subdivisions	
50		Campylobacter jejuni AL11116 16S 23S	58 39249-40761 41568-44457

30

Helicobacter pylori 26695 NC_000915

HPrmB16S 1511137-1512634 HPrmB23S 1473918-1476893

5 g. Cyanobacteria

Synechocystis sp. PCC 6803 NC 000911

Rm16Sa 2452187-2453675 Rm23Sa 2448839-2451721

Synechococcus sp. (Anacystis nidulans)

16S X03538 (1432-2918) 23S X00512 (251-3126)

h. CFB/Green sulfer bacteria group

15

10

Porphyromonas gingivalis 16S (1474nt)

L16492

B. Eukaryotic rRNA

Targeted nucleic acids of the invention may also be one or more types of eukaryotic rRNAs. Eukaryotes include, but are not limited to: mammals, fish, birds, amphibians, fungi, and plants. The following provides sequences for some of these targeted nucleic acids. It is contemplated that other eukaryotic rRNA sequences can be readily obtained by one of ordinary skill in the art, and thus, the invention includes, but is not limited to, the sequences shown below.

25	Superkingdom <u>Eukaryota</u> (eucaryota Homo sapiens (human)	es)
	18S `	M10098
	18S	K03432
	18S	X03205
30	28S	M11167
	Mus muculus	
	18S	X00686
	28S	X00525
35		
	Rattus norvegicus	
	18S	M11188
	18S	X01117
40	Rattus norvegicus V01270.1	
	18S	1-1874
	28S	3862-8647

31

II. Isolation and/or Depletion System Nucleic Acids

The present invention concerns compositions comprising a nucleic acid or a nucleic acid analog in a system or kit to deplete, isolate, or separate a nucleic acid population from other nucleic acid populations, for which enrichment may be desirable. It concerns a bridging nucleic acid and a capture nucleic acid to deplete, isolate, or separate out a targeted nucleic acid, as discussed above.

A. Bridging Nucleic Acids

5

10

15

20

25

30

Bridging nucleic acids of the invention comprise a bridging region and a targeting region. As discussed in other sections, the location of these regions may be throughout the molecule, which may be of a variety of lengths. The bridging nucleic acid may comprise RNA, DNA, both, or analogs of either or both.

The bridging region comprises a sequence that is complementary to at least five contiguous nucleotides in the capture nucleic acid. It is contemplated that that this region may be a homogenous sequence, that is, have the same nucleotide repeated across its length, such as a repeat of A, C, G, T, or U residues. However, to avoid hybridizing with a poly-A tailed mRNA in a sample comprising eukaryotic nucleic acids, it is contemplated that most embodiments will not have a poly-U or poly-T bridging region when dealing with such samples having poly-A tailed RNA. In some embodiments, the bridging region is a poly-C region and the capture region is a poly-G region, or vice versa. In other embodiments, the bridging region will be a random sequence that is complementary to the capture region (or the capture region will be random and the bridging region will be complementary to it). In further embodiments, the bridging region will have a designed sequence that is not homopolymeric but that is complementary to the capture region or vice versa. Sequences may be determined empirically. In many embodiments, it is preferred that this will be a random sequence or a defined sequence that is not a homopolymer. Some sequences will be determined empirically during evaluation in the assay.

B. Capture Nucleic Acids

Capture nucleic acids of the invention comprise a capture region and a nonreacting structure that allows the capture nucleic acid, any molecules specifically binding or hybridizing to the capture nucleic acid—such as the bridging nucleic acid—and any molecules specifically binding or hybridizing to the bridging nucleic acid—such as the targeted nucleic acid—to be isolated away from other nucleic acid populations.

The capture nucleic acid may comprise RNA, DNA, both, or analogs of either or both. However, in some embodiments of the invention, it is specifically contemplated to be homopolymeric (only one type of nucleotide residue in molecule, such as poly-C), though in other embodiments, it is specifically contemplated not to be homopolymeric and be heteropolymeric, as described for bridging regions.

1. Capture Regions

The main requirement for bridging and capture nucleic acid sequences is that they are complementary to one another. The capture region may be a poly-pyrimidine or poly-purine region comprising at least 5 nucleic acid residues. In addition, it may be heteropolymeric, either a random sequence or a designed sequence that is complementary to the bridging region of the nucleic acid with which it should hybridize.

In addition to the capture oligos already described herein, the following are also considered for use with the present invention:

NRS-5'-TAACCTGGTCGTAAC-3' (SEQ ID NO:87)

15

5

10

NRS-5'-CCCCCCCCCCCCC3' (SEQ ID NO:88)

NRS-5'-GCCCCTAACCTCGTCG (SEQ ID NO:89)

20 NRS-5'-CGGCCCTAGCCGGGTCGTACCTCCGG (SEQ ID NO:90)

NRS-5'-CGGCCCTAACCTGGTCGTAACTCCGG (SEQ ID NO:91)

NRS-5'-AGGCTTCGATCCCGGGATCCGCG (SEQ ID NO:92)

25

30

As discussed below, "NRS" refers to a non-reacting structure.

2. Nonreacting Structures (NRS)

A nonreacting structure is a compound or structure that will not react chemically with nucleic acids, and in some embodiments, with any molecule that may be in a sample. Nonreacting structures may comprise plastic, glass, teflon, silica, a magnet, a metal such as gold, carbon, cellulose, latex, polystyrene, and other synthetic polymers, nylon, cellulose, nitrocellulose, polymethacrylate, polyvinylchloride, styrene-divinylbenzene, or any chemically-

33

modified plastic. They may also be porous or non-porous materials. The structure may also be a particle of any shape that allows the targeted nucleic acid to be isolated, depleted, or separated. It may be a sphere, such as a bead, or a rod, or a flat-shaped structure, such as a plate with wells. Also, it is contemplated that the structure may be isolated by physical means or electromagnetic means. For example, a magnetic field may be used to attract a non-reacting structure that includes a magnet. The magnetic field may be in a stand or it may simply be placed on the side of a tube with the sample and a capture nucleic acid that is magnetized. Examples of physical ways to separate nucleic acids with their specifically hybridizing compounds are well known to those of skill in the art. A basket or other filter means may be employed to separate the capture nucleic acid and its hybridizing compounds (direct and indirect). The non-reacting structure and sample with nucleic acids of the invention may be centrifuged, filtered, dialyzed, or captured (with a magnet). When the structure is centrifuged it may be pelleted or passed through a centrifugible filter apparatus. The structure may also be filtered, including filtration using a pressure-driven system. Many such structures are available commercially and may be utilized herewith. Other examples can be found in WO 86/05815, WO90/06045, U.S. Patent 5,945,525, all of which are specifically incorporated by reference.

5

10

15

20

25

30

Cellulose is a structural polymer derived from vascular plants. Chemically, it is a linear polymer of the monosaccharide glucose, using β , 1-4 linkages. Cellulose can be provided commercially, including from the Whatman company, and can be chemically sheared or chemically modified to create preparations of a more fibrous or particulate nature. CF-1 cellulose from Whatman is an example that can be implemented in the present invention.

Synthetic plastic or glass beads may be employed in the context of the invention. The beads may be complexed with avidin or streptavidin and they may also be magnetized. The complexed streptavidin can be used to capture biotin linked to an oligo-dT or -U or poly (dT) or poly(U) moiety, either before or after hybridization to the poly(A) tails of mRNA. Alternatively, the oligo/poly(dT/U) moiety can be attached to the beads directly through chemical coupling. The beads may be collected using gravity- or pressure-based systems and/or filtration devices. If the beads are magnetized, a magnet can be used to separate the beads from the rest of the sample. The magnet may be employed with a stand or a stick or other type of physical structure to facilitate isolation.

34

Other components include isolation apparatuses such as filtration devices, including spin filters or spin columns.

C. Nucleic Acid Compositions

5

10

15

20

25

30

Embodiments of the present invention concern bridging, capture, and targeted nucleic acids. In particular aspects, a targeted nucleic acid encodes for or comprises a transcribed nucleic acid. In other aspects, a bridging nucleic acid comprises a targeting region that comprises a nucleic acid segment having the sequence of all or part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEO ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEO ID NO:15, SEO ID NO:16, SEO ID NO:17, SEO ID NO:18, SEO ID NO:19, SEO ID NO:20, SEO ID NO:21, SEO ID NO:22, SEO ID NO:23, SEO ID NO:24, SEO ID NO:25, SEO ID NO:26, SEO ID NO:27, SEO ID NO:28, SEO ID NO:29, SEO ID NO:30, SEO ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEO ID NO:49, SEO ID NO:50, SEO ID NO:51, SEO ID NO:52, SEO ID NO:53, SEO ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEO ID NO:60, SEO ID NO:61, SEO ID NO:62, SEO ID NO:63, SEO ID NO:64, SEO ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:72, or SEQ ID NO:73 (collectively referred to as "SEQ ID NOS:1-73"), as well as SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEO ID NO:86, SEO ID NO:87, SEO ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NOS:1-92"). In particular aspects, a targeted nucleic acid encodes a protein, polypeptide, peptide. Nucleic acids of the invention comprise RNA, DNA, analogs of RNA, analogs of DNA, or a combination thereof.

The term "nucleic acid" is well known in the art. A "nucleic acid" as used herein will generally refer to a molecule (i.e., a strand) of DNA, RNA or a derivative or analog thereof, comprising a nucleobase. A nucleobase includes, for example, a naturally occurring purine or pyrimidine base found in DNA (e.g., an adenine "A," a guanine "G," a thymine "T" or a cytosine "C") or RNA (e.g., an A, a G, an uracil "U" or a C). The term "nucleic acid" encompass the

terms "oligonucleotide" and "polynucleotide," each as a subgenus of the term "nucleic acid." The term "oligonucleotide" refers to a molecule of between about 3 and about 100 nucleobases in length. The term "polynucleotide" refers to at least one molecule of greater than about 100 nucleobases in length.

These definitions generally refer to a single-stranded molecule, but in specific embodiments will also encompass an additional strand that is partially, substantially or fully complementary to the single-stranded molecule. Thus, a nucleic acid may encompass a doublestranded molecule or a triple-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence comprising a molecule. As used herein, a single stranded nucleic acid may be denoted by the prefix "ss," a double stranded nucleic acid by the prefix "ds," and a triple stranded nucleic acid by the prefix "ts."

1. **Nucleobases**

5

10

15

20

25

30

As used herein a "nucleobase" refers to a heterocyclic base, such as for example a naturally occurring nucleobase (i.e., an A, T, G, C or U) found in at least one naturally occurring nucleic acid (i.e., DNA and RNA), and naturally or non-naturally occurring derivative(s) and analogs of such a nucleobase. A nucleobase generally can form one or more hydrogen bonds ("anneal" or "hybridize") with at least one naturally occurring nucleobase in manner that may substitute for naturally occurring nucleobase pairing (e.g., the hydrogen bonding between A and T, G and C, and A and U).

"Purine" and/or "pyrimidine" nucleobase(s) encompass naturally occurring purine and/or pyrimidine nucleobases and also derivative(s) and analog(s) thereof, including but not limited to, those of a purine or pyrimidine substituted by one or more of an alkyl, caboxyalkyl, amino, hydroxyl, halogen (i.e., fluoro, chloro, bromo, or iodo), thiol or alkylthiol moiety. Preferred alkyl (e.g., alkyl, caboxyalkyl, etc.) moieties comprise of from about 1, about 2, about 3, about 4, about 5, to about 6 carbon atoms. Other non-limiting examples of a purine or pyrimidine include a deazapurine, a 2,6-diaminopurine, a 5-fluorouracil, a xanthine, a hypoxanthine, a 8bromoguanine, a 8-chloroguanine, a bromothymine, a 8-aminoguanine, a 8-hydroxyguanine, a 8methylguanine, a 8-thioguanine, an azaguanine, a 2-aminopurine, a 5-ethylcytosine, a 5methylcyosine, a 5-bromouracil, a 5-ethyluracil, a 5-iodouracil, a 5-chlorouracil, a 5propyluracil, a thiouracil, a 2-methyladenine, a methylthioadenine, a N,N-diemethyladenine, an azaadenines, a 8-bromoadenine, a 8-hydroxyadenine, a 6-hydroxyaminopurine, a 6-thiopurine, a

4-(6-aminohexyl/cytosine), and the like. A table of non-limiting, purine and pyrimidine derivatives and analogs is also provided herein below.

Table 1-Purine and Pyrimidine Derivatives or Analogs			
Abbr.	Modified base description	Abbr.	Modified base description
ac4c	4-acetylcytidine	Mam5s2u	5-methoxyaminomethyl-2- thiouridine
Chm5u	5-(carboxyhydroxylmethyl) uridine	Man q	Beta,D-mannosylqueosine
Cm	2'-O-methylcytidine	Mcm5s2u	5-methoxycarbonylmethyl-2- thiouridine
Cmnm5s2u	5-carboxymethylamino-methyl-2- thioridine	Mcm5u	5- methoxycarbonylmethyluridi ne
Cmnm5u	5- carboxymethylaminomethyluridin e	Mo5u	5-methoxyuridine
D	Dihydrouridine	Ms2i6a	2-methylthio-N6- isopentenyladenosine
Fm	2'-O-methylpseudouridine	Ms2t6a	N-((9-beta-D-ribofuranosyl- 2-methylthiopurine-6- yl)carbamoyl)threonine
Gal q	Beta,D-galactosylqueosine	Mt6a	N-((9-beta-D- ribofuranosylpurine-6-yl)N- methyl-carbamoyl)threonine
Gm	2'-O-methylguanosine	Mv	Uridine-5-oxyacetic acid methylester
Ī	Inosine	o5u	Uridine-5-oxyacetic acid (v)
I6a	N6-isopentenyladenosine	Osyw	Wybutoxosine
mla	1-methyladenosine	P	Pseudouridine

Table 1-Purine and Pyrimidine Derivatives or Analogs			
Abbr.	Modified base description	Abbr.	Modified base description
mlf	1-methylpseudouridine	Q	Queosine
mlg	1-methylguanosine	s2c	2-thiocytidine
mlI	1-methylinosine	s2t	5-methyl-2-thiouridine
m22g	2,2-dimethylguanosine	s2u	2-thiouridine
m2a	2-methyladenosine	s4u	4-thiouridine
m2g	2-methylguanosine	T	5-methyluridine
m3c	3-methylcytidine	t6a	N-((9-beta-D-
			ribofuranosylpurine-6-
			yl)carbamoyl)threonine
m5c	5-methylcytidine	Tm	2'-O-methyl-5-methyluridine
m6a	N6-methyladenosine	Um	2'-O-methyluridine
m7g	7-methylguanosine	Yw	Wybutosine
Mam5u	5-methylaminomethyluridine	Х	3-(3-amino-3-
			carboxypropyl)uridine,
			(acp3)u

A nucleobase may be comprised of a nucleoside or nucleotide, using any chemical or natural synthesis method described herein or known to one of ordinary skill in the art.

2. Nucleosides

5

10

As used herein, a "nucleoside" refers to an individual chemical unit comprising a nucleobase covalently attached to a nucleobase linker moiety. A non-limiting example of a "nucleobase linker moiety" is a sugar comprising 5-carbon atoms (i.e., a "5-carbon sugar"), including but not limited to a deoxyribose, a ribose, an arabinose, or a derivative or an analog of a 5-carbon sugar. Non-limiting examples of a derivative or an analog of a 5-carbon sugar include a 2'-fluoro-2'-deoxyribose or a carbocyclic sugar where a carbon is substituted for an oxygen atom in the sugar ring.

Different types of covalent attachment(s) of a nucleobase to a nucleobase linker moiety are known in the art. By way of non-limiting example, a nucleoside comprising a purine (i.e., A

or G) or a 7-deazapurine nucleobase typically covalently attaches the 9 position of a purine or a 7-deazapurine to the 1'-position of a 5-carbon sugar. In another non-limiting example, a nucleoside comprising a pyrimidine nucleobase (i.e., C, T or U) typically covalently attaches a 1 position of a pyrimidine to a 1'-position of a 5-carbon sugar.

3. **Nucleotides**

5

10

15

20

25

30

As used herein, a "nucleotide" refers to a nucleoside further comprising a "backbone moiety". A backbone moiety generally covalently attaches a nucleotide to another molecule comprising a nucleotide, or to another nucleotide to form a nucleic acid. The "backbone mojety" in naturally occurring nucleotides typically comprises a phosphorus moiety, which is covalently attached to a 5-carbon sugar. The attachment of the backbone moiety typically occurs at either the 3'- or 5'-position of the 5-carbon sugar. However, other types of attachments are known in the art, particularly when a nucleotide comprises derivatives or analogs of a naturally occurring 5-carbon sugar or phosphorus moiety.

4. **Nucleic Acid Analogs**

A nucleic acid may comprise, or be composed entirely of, a derivative or analog of a nucleobase, a nucleobase linker moiety and/or backbone moiety that may be present in a naturally occurring nucleic acid. As used herein a "derivative" refers to a chemically modified or altered form of a naturally occurring molecule, while the terms "mimic" or "analog" refer to a molecule that may or may not structurally resemble a naturally occurring molecule or moiety, but possesses similar functions. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure. Nucleobase, nucleoside and nucleotide analogs or derivatives are well known in the art, and have been described (see for example, Scheit, 1980, incorporated herein by reference).

Additional non-limiting examples of nucleosides, nucleotides or nucleic acids comprising 5-carbon sugar and/or backbone moiety derivatives or analogs, include those in U.S. Patent No. 5,681,947 which describes oligonucleotides comprising purine derivatives that form triple helixes with and/or prevent expression of dsDNA; U.S. Patents 5,652,099 and 5,763,167 which describe nucleic acids incorporating fluorescent analogs of nucleosides found in DNA or RNA, particularly for use as fluorescent nucleic acids probes; U.S. Patent 5,614,617 which describes oligonucleotide analogs with substitutions on pyrimidine rings that possess enhanced nuclease stability; U.S. Patents 5,670,663, 5,872,232 and 5,859,221 which describe oligonucleotide

5

10

15

20

25

30

PCT/US02/41014

analogs with modified 5-carbon sugars (i.e., modified 2'-deoxyfuranosyl moieties) used in nucleic acid detection; U.S. Patent 5,446,137 which describes oligonucleotides comprising at least one 5-carbon sugar moiety substituted at the 4' position with a substituent other than hydrogen that can be used in hybridization assays; U.S. Patent 5,886,165 which describes oligonucleotides with both deoxyribonucleotides with 3'-5' internucleotide linkages and ribonucleotides with 2'-5' internucleotide linkages; U.S. Patent 5,714,606 which describes a modified internucleotide linkage wherein a 3'-position oxygen of the internucleotide linkage is replaced by a carbon to enhance the nuclease resistance of nucleic acids; U.S. Patent 5,672,697 which describes oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages that enhance nuclease resistance; U.S. Patents 5,466,786 and 5,792,847 which describe the linkage of a substituent moiety, which may comprise a drug or label to the 2' carbon of an oligonucleotide to provide enhanced nuclease stability and ability to deliver drugs or detection moieties; U.S. Patent 5,223,618 which describes oligonucleotide analogs with a 2 or 3 carbon backbone linkage attaching the 4' position and 3' position of adjacent 5-carbon sugar mojety to enhanced cellular uptake, resistance to nucleases and hybridization to target RNA; U.S. Patent 5,470,967 which describes oligonucleotides comprising at least one sulfamate or sulfamide internucleotide linkage that are useful as nucleic acid hybridization probe; U.S. Patents 5,378,825, 5,777,092, 5,623,070, 5,610,289 and 5,602,240 which describe oligonucleotides with three or four atom linker moiety replacing phosphodiester backbone moiety used for improved nuclease resistance, cellular uptake and regulating RNA expression; U.S. Patent 5,858,988 which describes hydrophobic carrier agent attached to the 2'-O position of oligonucleotides to enhanced their membrane permeability and stability; U.S. Patent 5,214,136, which describes oligonucleotides conjugated to anthraquinone at the 5' terminus that possess enhanced hybridization to DNA or RNA; enhanced stability to nucleases; U.S. Patent 5,700,922 which describes PNA-DNA-PNA chimeras wherein the DNA comprises 2'-deoxy-erythropentofuranosyl nucleotides for enhanced nuclease resistance, binding affinity, and ability to activate RNase H; and U.S. Patent 5,708,154 which describes RNA linked to a DNA to form a DNA-RNA hybrid. Other analogs that may be used with compositions of the invention include U.S. Patent 5,216,141 (discussing oligonucleotide analogs containing sulfur linkages), U.S. Patent 5,432,272 (concerning oligonucleotides having nucleotides with heterocyclic bases), and U.S. Patents 6,001,983, 6,037,120, 6,140,496 (involving oligonucleotides with non-standard bases), all of which are incorporated by reference.

5

10

15

20

25

30

5. Polyether and Peptide Nucleic Acids and Locked Nucleic Acids

In certain embodiments, it is contemplated that a nucleic acid comprising a derivative or analog of a nucleoside or nucleotide may be used in the methods and compositions of the invention. A non-limiting example is a "polyether nucleic acid", described in U.S. Patent Serial No. 5,908,845, incorporated herein by reference. In a polyether nucleic acid, one or more nucleobases are linked to chiral carbon atoms in a polyether backbone.

Another non-limiting example is a "peptide nucleic acid", also known as a "PNA", "peptide-based nucleic acid analog" or "PENAM", described in U.S. Patent Serial Nos. 5,786,461, 5891,625, 5,773,571, 5,766,855, 5,736,336, 5,719,262, 5,714,331, 5,539,082, and WO 92/20702, each of which is incorporated herein by reference. Peptide nucleic acids generally have enhanced sequence specificity, binding properties, and resistance to enzymatic degradation in comparison to molecules such as DNA and RNA (Egholm et al., 1993; PCT/EP/01219). A peptide nucleic acid generally comprises one or more nucleotides or nucleosides that comprise a nucleobase moiety, a nucleobase linker moiety that is not a 5-carbon sugar, and/or a backbone moiety that is not a phosphate backbone moiety. Examples of nucleobase linker moieties described for PNAs include aza nitrogen atoms, amido and/or ureido tethers (see for example, U.S. Patent No. 5,539,082). Examples of backbone moieties described for PNAs include an aminoethylglycine, polyamide, polyethyl, polythioamide, polysulfinamide or polysulfonamide backbone moiety.

In certain embodiments, a nucleic acid analogue such as a peptide nucleic acid may be used to inhibit nucleic acid amplification, such as in PCR, to reduce false positives and discriminate between single base mutants, as described in U.S. Patent Serial No. 5,891,625. Other modifications and uses of nucleic acid analogs are known in the art, and are encompassed by the bridging and capture nucleic acids of the invention. In a non-limiting example, U.S. Patent 5,786,461 describes PNAs with amino acid side chains attached to the PNA backbone to enhance solubility of the molecule. In another example, the cellular uptake property of PNAs is increased by attachment of a lipophilic group. Several alkylamino moieties used to enhance cellular uptake of a PNA are described in U.S. Patent Nos. 5,766,855, 5,719,262, 5,714,331 and 5,736,336, which describe PNAs comprising naturally and non-naturally occurring nucleobases and alkylamine side chains that provide improvements in sequence specificity, solubility and/or binding affinity relative to a naturally occurring nucleic acid.

WO 03/054162

5

10

15

20

25

30

Another non-limiting example is a locked nucleic acid or "LNA." An LNA monomer is a bicyclic compound that is structurally similar to RNA nucleosides. LNAs have a furanose conformation that is restricted by a methylene linker that connects the 2'-O position to the 4'-C position, as described in Koshkin *et al.*, 1998a and 1998b and Wahlestedt *et al.*, 2000.

6. Preparation of Nucleic Acids

A nucleic acid may be made by any technique known to one of ordinary skill in the art, such as for example, chemical synthesis, enzymatic production or biological production. Non-limiting examples of a synthetic nucleic acid (e.g., a synthetic oligonucleotide), include a nucleic acid made by in vitro chemical synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266,032, incorporated herein by reference, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., 1986 and U.S. Patent No. 5,705,629, each incorporated herein by reference. In the methods of the present invention, one or more oligonucleotide may be used. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents. 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

A non-limiting example of an enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCRTM (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195, each incorporated herein by reference), or the synthesis of an oligonucleotide described in U.S. Patent No. 5,645,897, incorporated herein by reference. A non-limiting example of a biologically produced nucleic acid includes a recombinant nucleic acid produced (i.e., replicated) in a living cell, such as a recombinant DNA vector replicated in bacteria (see for example, Sambrook et al. 1989, incorporated herein by reference).

7. Purification of Nucleic Acids

A nucleic acid may be purified on polyacrylamide gels, cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook *et al.*, 1989, incorporated herein by reference).

In certain aspect, the present invention concerns a nucleic acid that is an isolated nucleic acid. As used herein, the term "isolated nucleic acid" refers to a nucleic acid molecule (e.g., an RNA or DNA molecule) that has been isolated free of, or is otherwise free of, the bulk of the

WO 03/054162

5

10

20

25

30

total genomic and transcribed nucleic acids of one or more cells. In certain embodiments, "isolated nucleic acid" refers to a nucleic acid that has been isolated free of, or is otherwise free of, bulk of cellular components or in vitro reaction components such as for example, macromolecules such as lipids or proteins, small biological molecules, and the like.

8. Nucleic Acid Segments

In certain embodiments, the nucleic acid comprises a nucleic acid segment. As used herein, the term "nucleic acid segment," are smaller fragments of a nucleic acid, such as for non-limiting example, those that correspond to targeted, targeting, bridging, and capture regions. Thus, a "nucleic acid segment" may comprise any part of a gene sequence, of from about 2 nucleotides to the full length of a targeted nucleic acid, capture nucleic acid, or bridging nucleic acid.

Various nucleic acid segments may be designed based on a particular nucleic acid sequence, and may be of any length. By assigning numeric values to a sequence, for example, the first residue is 1, the second residue is 2, etc., an algorithm defining all nucleic acid segments can be created:

15 n to n + y

where n is an integer from 1 to the last number of the sequence and y is the length of the nucleic acid segment minus one, where n + y does not exceed the last number of the sequence. Thus, for a 10-mer, the nucleic acid segments correspond to bases 1 to 10, 2 to 11, 3 to 12 ... and so on. For a 15-mer, the nucleic acid segments correspond to bases 1 to 15, 2 to 16, 3 to 17 ... and so on. For a 20-mer, the nucleic segments correspond to bases 1 to 20, 2 to 21, 3 to 22 ... and so on. In certain embodiments, the nucleic acid segment may be a probe or primer. As used herein, a "probe" generally refers to a nucleic acid used in a detection method or composition. As used herein, a "primer" generally refers to a nucleic acid used in an extension or amplification method or composition.

9. Nucleic Acid Complements

The present invention also encompasses a nucleic acid that is complementary to a other nucleic acids of the invention and targeted nucleic acids. More specifically, a targeting region in a bridging nucleic acid is complementary to the targeted region of the targeted nucleic acid and a bridging region of the bridging nucleic acid is complementary to a capture region of a capture nucleic acid. In particular embodiments the invention encompasses a nucleic acid or a nucleic

acid segment identical or complementary to all or part of the sequences set forth in SEQ ID NOS:1-92. A nucleic acid is "complement(s)" or is "complementary" to another nucleic acid when it is capable of base-pairing with another nucleic acid according to the standard Watson-Crick, Hoogsteen or reverse Hoogsteen binding complementarity rules. Unless otherwise specified, a nucleic acid region is "complementary" to another nucleic acid region if there is at least 70, 80%, 90% or 100% Watson-Crick base-pairing (A:T or A:U, C:G) between or between at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500 or more contiguous nucleic acid bases of the regions. As used herein "another nucleic acid" may refer to a separate molecule or a spatial separated sequence of the same molecule.

As used herein, the term "complementary" or "complement(s)" also refers to a nucleic acid comprising a sequence of consecutive nucleobases or semiconsecutive nucleobases (e.g., one or more nucleobase moieties are not present in the molecule) capable of hybridizing to another nucleic acid strand or duplex even if less than all the nucleobases do not base pair with a counterpart nucleobase. In certain embodiments, a "complementary" nucleic acid comprises a sequence in which at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, and any range derivable therein, of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic acid molecule during hybridization, as described in the Examples. In certain embodiments, the term "complementary" refers to a nucleic acid that may hybridize to another nucleic acid strand or duplex under conditions described in the Examples, as would be understood by one of ordinary skill in the art.

In certain embodiments, a "partly complementary" nucleic acid comprises a sequence that may hybridize in low stringency conditions to a single or double stranded nucleic acid, or contains a sequence in which less than about 70% of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic acid molecule during hybridization.

10. Hybridization

5

10

15

20

25

30

As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or

triple stranded nature. The term "anneal" as used herein is synonymous with "hybridize." The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

5

10

15

20

25

30

44

As used herein "stringent condition(s)" or "high stringency" are those conditions that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating a nucleic acid, such as a gene or a nucleic acid segment thereof, or detecting at least one specific mRNA transcript or a nucleic acid segment thereof, and the like.

Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. Alternatively, stringent conditions may be determined largely by temperature in the presence of a TMAC solution with a defined molarity such as 3M TMAC. For example, in 3 M TMAC, stringent conditions include the following: for complementary nucleic acids with a length of 15 bp, a temperature of 45 °C to 55 °C; for complementary nucleotides with a length of 27 bases, a temperature of 65 °C to 75 °C; and, for complementary nucleotides with a length of >200 nucleotides, a temperature of 90 °C to 95°C. The publication of Wood *et al.*, 1985, which is specifically incorporated by reference, provides examples of these parameters. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

It is also understood that these ranges, compositions and conditions for hybridization are mentioned by way of non-limiting examples only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to employ varying conditions of hybridization to achieve varying degrees of selectivity of a nucleic acid towards a target sequence. In a non-limiting example, identification or isolation of a related target nucleic

45

acid that does not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions", and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M NaCl at a temperature range of about 20°C to about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suite a particular application.

11. Oligonucleotide Synthesis

5

10

15

20

25

Oligonucleotide synthesis is performed according to standard methods. See, for example, Itakura and Riggs (1980). Additionally, U.S. Patent 4,704,362; U.S. Patent 5,221,619, U.S. Patent . 5,583,013 each describe various methods of preparing synthetic structural genes.

Oligonucleotide synthesis is well known to those of skill in the art. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents. 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

Basically, chemical synthesis can be achieved by the diester method, the triester method polynucleotides phosphorylase method and by solid-phase chemistry. These methods are discussed in further detail below.

Diester method. The diester method was the first to be developed to a usable state, primarily by Khorana and co-workers. (Khorana, 1979). The basic step is the joining of two suitably protected deoxynucleotides to form a dideoxynucleotide containing a phosphodiester bond. The diester method is well established and has been used to synthesize DNA molecules (Khorana, 1979).

Triester method. The main difference between the diester and triester methods is the presence in the latter of an extra protecting group on the phosphate atoms of the reactants and products (Itakura et al., 1975). The phosphate protecting group is usually a chlorophenyl group, which renders the nucleotides and polynucleotide intermediates soluble in organic solvents. Therefore purification's are done in chloroform solutions. Other improvements in the method include (i) the block coupling of trimers and larger oligomers, (ii) the extensive use of high-

performance liquid chromatography for the purification of both intermediate and final products, and (iii) solid-phase synthesis.

Polynucleotide phosphorylase method. This is an enzymatic method of DNA synthesis that can be used to synthesize many useful oligodeoxynucleotides (Gillam et al., 1978; Gillam et al., 1979). Under controlled conditions, polynucleotide phosphorylase adds predominantly a single nucleotide to a short oligodeoxynucleotide. Chromatographic purification allows the desired single adduct to be obtained. At least a trimer is required to start the procedure, and this primer must be obtained by some other method. The polynucleotide phosphorylase method works and has the advantage that the procedures involved are familiar to most biochemists.

Solid-phase methods. Drawing on the technology developed for the solid-phase synthesis of polypeptides, it has been possible to attach the initial nucleotide to solid support material and proceed with the stepwise addition of nucleotides. All mixing and washing steps are simplified, and the procedure becomes amenable to automation. These syntheses are now routinely carried out using automatic DNA synthesizers.

Phosphoramidite chemistry (Beaucage, and Lyer, 1992) has become by far the most widely used coupling chemistry for the synthesis of oligonucleotides. As is well known to those skilled in the art, phosphoramidite synthesis of oligonucleotides involves activation of nucleoside phosphoramidite monomer precursors by reaction with an activating agent to form activated intermediates, followed by sequential addition of the activated intermediates to the growing oligonucleotide chain (generally anchored at one end to a suitable solid support) to form the oligonucleotide product.

12. Expression Vectors

5

10

15

20

25

Other ways of creating nucleic acids of the invention include the use of a recombinant vector created through the application of recombinant nucleic acid technology known to those of skill in the art or as described herein. A recombinant vector may comprise a bridging or capture nucleic acid, particularly one that is a polynucleotide, as opposed to an oligonucleotide. An expression vector can be used create nucleic acids that are lengthy, for example, containing multiple targeting regions or relatively lengthy targeting regions, such as those greater than 100 residues in length.

5

10

15

20

25

The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (see, for example, Sambrook et al., 2001 and Ausubel et al., 1994, both incorporated herein by reference).

47

The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for a RNA capable of being transcribed. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operable linked coding sequence in a particular host cell. In addition to control sequences that govern transcription (promoters and enhancers) and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well that are well known to those of skill in the art, such as screenable and selectable markers, ribosome binding site, multiple cloning sites, splicing sites, poly A sequences, origins of replication, and other sequences that allow expression in different hosts.

Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with the present invention to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Many such systems are commercially and widely available.

The nucleotide and protein, polypeptide and peptide sequences for various genes have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. For example, the nucleotide sequences of rRNAs of various organisms are readily available. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (http://www.ncbi.nlm.nih.gov/). The coding regions for all or part of these known genes may be amplified and/or expressed using the techniques disclosed herein or by any technique that would be know to those of ordinary skill in the art.

48

13. Nucleic Acid Arrays

5

10

15

20

25

30

Because the present invention provides efficient methods of enriching in mRNA, which can be used to make cDNA, the present invention extends to the use of cDNAs with arrays. The term "array" as used herein refers to a systematic arrangement of nucleic acid. For example, a cDNA population that is representative of a desired source (e.g., human adult brain) is divided up into the minimum number of pools in which a desired screening procedure can be utilized to detect a cDNA and which can be distributed into a single multi-well plate. Arrays may be of an aqueous suspension of a cDNA population obtainable from a desired mRNA source, comprising: a multi-well plate containing a plurality of individual wells, each individual well containing an aqueous suspension of a different content of a cDNA population. The cDNA population may include cDNA of a predetermined size. Furthermore, the cDNA population in all the wells of the plate may be representative of substantially all mRNAs of a predetermined size from a source. Examples of arrays, their uses, and implementation of them can be found in U.S. Patent Nos. 6,329,209, 6,329,140, 6,324,479, 6,322,971, 6,316,193, 6,309,823, 5,412,087, 5,445,934, and 5,744,305, which are herein incorporated by reference.

The number of cDNA clones array on a plate may vary. For example, a population of cDNA from a desired source can have about 200,000-6,000,000 cDNAs, about 200,000-2,000,000, 300,000-700,000, about 400,000-600,000, or about 500,000 cDNAs, and combinations thereof. Such a population can be distributed into a small set of multi-well plates, such as a single 96-well plate or a single 384-well plate. For instance, when about 1000-10,000 cDNAs, preferably about 3,500-7,000, more preferably about 5,000, from a population are present in a single well of a 96-well or 384-well plate, PCR can be utilized to clone a single, target gene using a set of primers.

The term a "nucleic acid array" refers to a plurality of target elements, each target element comprising one or more nucleic acid molecules immobilized on one or more solid surfaces to which sample nucleic acids can be hybridized. The nucleic acids of a target element can contain sequence(s) from specific genes or clones, e.g. from the regions identified here. Other target elements will contain, for instance, reference sequences. Target elements of various dimensions can be used in the arrays of the invention. Generally, smaller, target elements are preferred. Typically, a target element will be less than about 1 cm in diameter. Generally element sizes are from 1 μ m to about 3 mm, between about 5 μ m and about 1 mm. The target elements

5

10

15

20

25

30

of the arrays may be arranged on the solid surface at different densities. The target element densities will depend upon a number of factors, such as the nature of the label, the solid support, and the like. One of skill will recognize that each target element may comprise a mixture of nucleic acids of different lengths and sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths. The length and complexity of the nucleic acid fixed onto the target element is not critical to the invention. One of skill can adjust these factors to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations. In various embodiments, target element sequences will have a complexity between about 1 kb and about 1 Mb, between about 10 kb to about 500 kb, between about 200 to about 500 kb, and from about 50 kb to about 150 kb.

PCT/US02/41014

Microarrays are known in the art and consist of a surface to which probes that correspond in sequence to gene products (e.g., cDNAs, mRNAs, cRNAs, polypeptides, and fragments thereof), can be specifically hybridized or bound at a known position. In one embodiment, the microarray is an array (i.e., a matrix) in which each position represents a discrete binding site for a product encoded by a gene (e.g., a protein or RNA), and in which binding sites are present for products of most or almost all of the genes in the organism's genome. In a preferred embodiment, the "binding site" (hereinafter, "site") is a nucleic acid or nucleic acid analogue to which a particular cognate cDNA can specifically hybridize. The nucleic acid or analogue of the binding site can be, e.g., a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

A microarray may contains binding sites for products of all or almost all genes in the target organism's genome, but such comprehensiveness is not necessarily required. Usually the microarray will have binding sites corresponding to at least about 50% of the genes in the genome, often at least about 75%, more often at least about 85%, even more often more than about 90%, and most often at least about 99%. Preferably, the microarray has binding sites for genes relevant to the action of a drug of interest or in a biological pathway of interest. A "gene" is identified as an open reading frame (ORF) of preferably at least 50, 75, or 99 amino acids from which a messenger RNA is transcribed in the organism (e.g., if a single cell) or in some cell in a multicellular organism. The number of genes in a genome can be estimated from the number

WO 03/054162 PCT/US02/41014 50

of mRNAs expressed by the organism, or by extrapolation from a well-characterized portion of the genome. When the genome of the organism of interest has been sequenced, the number of ORFs can be determined and mRNA coding regions identified by analysis of the DNA sequence.

The nucleic acid or analogue are attached to a solid support, which may be made from glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena et al., 1995a. See also DeRisi et al., 1996; Shalon et al., 1996; Schena et al., 1995b. Each of these articles is incorporated by reference in its entirety.

5

10

15

20

25

30

Other methods for making microarrays, e.g., by masking (Maskos et al., 1992), may also be used. In principal, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook et al., 1989, which is incorporated in its entirety for all purposes), could be used, although, as will be recognized by those of skill in the art, very small arrays will be preferred because hybridization volumes will be smaller.

Labeled cDNA is prepared from mRNA by oligo dT-primed or random-primed reverse transcription, both of which are well known in the art (see e.g., Klug et al., 1987). Reverse transcription may be carried out in the presence of a dNTP conjugated to a detectable label, most preferably a fluorescently labeled dNTP. Alternatively, isolated mRNA can be converted to labeled antisense RNA synthesized by in vitro transcription of double-stranded cDNA in the presence of labeled dNTPs (Lockhart et al., 1996, which is incorporated by reference in its entirety for all purposes). In alternative embodiments, the cDNA or RNA probe can be synthesized in the absence of detectable label and may be labeled subsequently, e.g., by incorporating biotinylated dNTPs or rNTP, or some similar means (e.g., photo-cross-linking a psoralen derivative of biotin to RNAs), followed by addition of labeled streptavidin (e.g., phycoerythrin-conjugated streptavidin) or the equivalent.

Fluorescently-labeled probes can be used, including suitable fluorophores such as fluorescein, lissamine, phycoerythrin, rhodamine (Perkin Elmer Cetus), Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, FluorX (Amersham) and others (see, e.g., Kricka, 1992). It will be appreciated that pairs of fluorophores are chosen that have distinct emission spectra so that they can be easily distinguished. In another embodiment, a label other than a fluorescent label is used. For example, a radioactive label, or a pair of radioactive labels with distinct emission spectra, can be used (see

51

Zhao et al., 1995; Pietu et al., 1996). However, because of scattering of radioactive particles, and the consequent requirement for widely spaced binding sites, use of radioisotopes is a less-preferred embodiment.

In one embodiment, labeled cDNA is synthesized by incubating a mixture containing 0.5 mM dGTP, dATP and dCTP plus 0.1 mM dTTP plus fluorescent deoxyribonucleotides (e.g., 0.1 mM Rhodamine 110 UTP (Perken Elmer Cetus) or 0.1 mM Cy3 dUTP (Amersham)) with reverse transcriptase (e.g., SuperScriptTM, Invitrogen Inc.) at 42°C for 60 min.

III. Methods for Isolating and Depleting Targeted Nucleic Acids

5

10

15

20

25

30

Methods of the invention involve preparing a sample comprising a targeted nucleic acid, preparing a bridging nucleic acid, preparing a capture nucleic acid, incubating the sample with the bridging nucleic acid, incubating the sample with a capture nucleic acid, incubating the bridging nucleic acid with the capture nucleic acid, incubating compounds under conditions allowing for hybridization among complementary regions, washing the sample and/or the capture and/or bridging nucleic acids, and isolating the capture nucleic acids and any accompanying compounds (compounds that bind or hybridize directly or indirectly to the capture nucleic acids). Steps of the invention are not required to be in a particular order and thus, the invention covers methods in which the order of the steps varies.

Hybridization conditions are discussed earlier. Wash conditions may involve temperatures between 20°C and 75°C, between 25°C and 70°C, between 30°C and 65°C, between 35°C and 60°C, between 40°C and 55°C, between 45°C and 50°C, or at temperatures within the ranges specified.

Buffer conditions for hybridization of nucleic acid compositions are well known to those of skill in the art. It is specifically contemplated that isostabilizing agents may be employed in hybridization and wash buffers in methods of the invention. U.S. Ser. No. 09/854,412 describes the use of tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC) in such buffers; this application is specifically incorporated by reference herein. The concentration of an isostabilizing agent in a hybridization (binding) buffer may be between about 1.0 M and about 5.0 M, is about 4.0 M, or is about 2.0 M. Also specifically contemplated is a wash solution with an isostabilizing agent concentration of between about 0.1 M and 3.0 M, including 0.1 M increments within the range. Wash buffers may or may not contain Tris. However, in

PCT/US02/41014 WO 03/054162 52

some embodiments of the invention, the wash solution consists of water and no other salts or buffers. In some embodiments of the invention, the hybridizing or wash buffer may include guanidinium isothiocyanate, though in some embodiments this chemical is specifically contemplated to be absent. The concentration of guanidinium may be between about 0.4 M and about 3.0 M

A solution or buffer to elute targeted nucleic acids from the hybridizing nucleic acids (indirect or direct) may be implemented in some kits and methods of the invention. The elution buffer or solution can be an aqueous solution lacking salt, such as TE or water. Elution may occur at room temperature or it may occur at temperatures between 15°C and 100°C, between 20°C and 95°C, between 25°C and 90°C, between 30°C and 85°C, between 35°C and 80°C, between 40°C and 75°C, between 45°C and 70°C, between 50°C and 65°C, between 55°C and 60°C, or at temperatures within the ranges specified.

Quantitation of RNA A.

5

10

15

20

25

1. Assessing RNA yield by UV absorbance

The concentration and purity of RNA can be determined by diluting an aliquot of the preparation (usually a 1:50 to 1:100 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA) or water, and reading the absorbance in a spectrophotometer at 260 nm and 280 nm.

An A_{260} of 1 is equivalent to 40 µg RNA/ml. The concentration (µg/ml) of RNA is therefore calculated by multiplying the A₂₆₀ X dilution factor X 40 µg/ml. The following is a typical example:

The typical yield from 10 μ g total RNA is 3 - 5 μ g. If the sample is re-suspended in 25 μ l, this means that the concentration will vary between 120 ng/ μ l and 200 ng/ μ l. One μ l of the prep is diluted 1:50 into 49 μ l of TE. The A₂₆₀ = 0.1. RNA concentration = 0.1 X 50 X 40 μ g/ml = 200 µg/ml or 0.2 µg/µl. Since there are 24 µl of the prep remaining after using 1 μ l to measure the concentration, the total amount of remaining RNA is 24 μ l X 0.2 μ g/ μ l = 4.8 μ g.

2. Assessing RNA yield with RiboGreen®

Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation can be employed to measure RNA concentration.

53

B. Denaturing Agarose Gel Electrophoresis

Many mRNAs form extensive secondary structure. Ribosomal RNA depletion may be evaluated by agarose gel electrophoresis. Because of this, it is best to use a denaturing gel system to analyze RNA samples. A positive control should be included on the gel so that any unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. RNA molecular weight markers, an RNA sample known to be intact, or both, can be used for this purpose. It is also a good idea to include a sample of the starting RNA that was used in the enrichment procedure.

Ambion's NorthernMaxTM reagents for Northern Blotting include everything needed for denaturing agarose gel electrophoresis. These products are optimized for ease of use, safety, and low background, and they include detailed instructions for use. An alternative to using the NorthernMax reagents is to use a procedure described in "Current Protocols in Molecular Biology", Section 4.9 (Ausubel et al., eds.), hereby incorporated by reference. It is more difficult and time-consuming than the Northern-Max method, but it gives similar results.

C. Agilent 2100 Bioanalyzer

5

10

15

20

25

1. Evaluating rRNA Removal with the RNA 6000 LabChip

An effective method for evaluating rRNA removal utilizes RNA analysis with the Caliper RNA 6000 LabChip Kit and the Agilent 2100 Bioanalayzer. Follow the instructions provided with the RNA 6000 LabChip Kit for RNA analysis. This system performs best with RNA solutions at concentrations between 50 and 250 ng/ μ l. Loading 1 μ l of a typical enriched RNA sample is usually adequate for good performance.

2. Expected Results

In enriched mRNA samples from prokaryotes, the 16S and 23S rRNA peaks will be absent or present in only very small amounts. The peak calling feature of the software may fail to identify the peaks containing small quantities of leftover 16S and 23S rRNAs. A peak corresponding to 5S and tRNAs may be present depending on how the total RNA was initially purified. If RNA was purified by a glass fiber filter method prior to enrichment, this peak will be smaller. The size and shape of the 5S rRNA-tRNA peak is unchanged by some embodiments.

PCT/US02/41014 WO 03/054162 54

IV. **KITS**

5

10

15

20

25

Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a bridging nucleic acid and a capture nucleic acid may be comprised in a kit. The kits will thus comprise, in suitable container means, a bridging nucleic acid and a capture nucleic of the present invention. It may also include one or more buffers, such as hybridization buffer or a wash buffer, compounds for preparing the sample, and components for isolating the capture nucleic acid via the nonreacting structure. Other kits of the invention may include components for making a nucleic acid array, and thus, may include, for example, a solid support.

The kits may comprise suitably aliquoted nucleic acid compositions of the present invention, whether labeled or unlabeled, as may be used to isolate, deplete, or separate a targeted nucleic acid. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit (bridging and capture nucleic acids may be packaged together), the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred.

However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the nucleic acid formulations are placed, 5

preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, e.g., injection and/or blow-molded plastic containers into which the desired vials are retained.

Such kits may also include components that facilitate isolation of the targeting molecule, such as filters, beads, or a magnetic stand. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or solution as well as for the targeting agent.

A kit will also include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented.

Kits of the invention may also include one or more of the following, in addition to a capture nucleic acid and a bridging nucleic acid:

- 1) Control RNA (E. coli or other appropriate RNA);
- 15 2) Nuclease-free water;
 - 3) RNase-free containers, such as 1.5 ml tubes;
 - 4) RNase-free elution tubes;
 - 5) glycogen;
 - 6) ethanol;
- 20 7) sodium acetate;
 - 8) ammonium acetate;
 - 9) magnetic stand or other magnetic field;
 - 10) agarose;
 - 11) nucleic acid size marker;
- 25 12) RNase-free tube tips;
 - 13) and RNase or DNase inhibitors.

IV. Examples

5

10

15

20

25

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Furthermore, these examples are provided as one of many ways of implementing the claimed method and using the compositions of the invention. It is contemplated that the invention is not limited to the specific conditions set forth below, but that the conditions below provide examples of how to implement the invention.

EXAMPLE 1:

Materials

The following materials were used in the methods described herein for the selective removal of 16S and 23S rRNA and/or 18S and 28S rRNA, and hence mRNA enrichment, from total RNA. All steps are performed at room temperature unless otherwise indicated.

1. Bridging Nucleic Acids

In the following examples, the bridging regions are the poly-A stretches in the respective oligonucleotides.

Targeting regions for prokaryotic 16S and 23S rRNAs were designed based on a sequence comparison of different rRNAs from different bacteria to *E. coli* rRNA with MegAlign sequence analysis software version 4.05 from DNA Star, Incorporated (FIG. 2). The targeting regions are shown, in the examples below, 3' of the bridging regions. Thus, the targeting region encompasses the remaining, non-bridging region of each molecule described below. SEQ ID NOs are provided for the targeting regions of the bridging nucleic acids provided below (*i.e.*, sequence of bridging regions not included in SEQ ID NO.).

16S prokaryotic rRNA bridging oligonucleotides

d16S-358 (SEQ ID NO:1)

	5'-AAAAAAAAAAAAAAAAACTGCTGCCTCCCGTAGGAGTCT-3'
	d16S-537 (SEQ ID NO:2)
5	5'-AAAAAAAAAAAAAAAACGTATTACCGCGGCTGCTGGCAC-3'
,	d16S-548 (SEQ ID NO:3)
	5'-AAAAAAAAAAAAAAAAACGCCCAGTAATTCCGATTAACGC-3'
	d16S-807 (SEQ ID NO:4)
10	5'-AAAAAAAAAAAAAAAAAATGGACTACCAGGGTATCTAATCC-3'
	d16S-1092 (SEQ ID NO:5)
	5'-AAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTGCGGGACTT-3'
15	d16S-3' (SEQ ID NO:6)
	5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	23S prokaryotic rRNA bridging oligonucleotides
	d23S-488 (SEQ ID NO:7)
20	5'-AAAAAAAAAAAAAAAAGGTTCTTTTCACTCCCCTCGCC-3'
	d23S-581 (SEQ ID NO:8)
	5'-AAAAAAAAAAAAAAAAAAAGACCCATTATACAAAAGGTACGC-3
25	d23S-1118 (SEQ ID NO:9)
	5'-AAAAAAAAAAAAAAAAGCCCCGTTACATCTTCCGCGCAG-3'
	d23S-1926 (SEQ ID NO:10)
30	5'-AAAAAAAAAAAAAAAAACGACAAGGAATTTCGCTACCTTA-3'
50	d23S-1954 (SEQ ID NO:11)
	5'-AAAAAAAAAAAAAAAAACTTACCCGACAAGGAATTTCGC-3'
	d23S-2511 (SEQ ID NO:12)
35	5'-AAAAAAAAAAAAAAAAAAGAGCCGACATCGAGGTGCCAAAC-3
	d23S-3' (SEQ ID NO:13)
	5'-AAAAAAAAAAAAAAAAAAGGTTAAGCCTCACGGTTCATT-3'
40	d23S-1704 (SEQ ID NO:15)
	5'-AAAAAAAAAAAAAAAACCCCTTCTCCCGAAGTTACGGGG-3'
	d23S-1105 (SEQ ID NO:16)
	5'-AAAAAAAAAAAAAAAAAAGTGAGCTATTACGCTTTCTTT-3'

WO 03/054162

58

RNA oligo bridging oligonucleotide

r23S-3' (SEQ ID NO:14)

5

Eukaryotic 18S rRNA bridging oligonucleotides

d18S-3711 (SEQ ID NO:17)

AAA AAA AAA AAA AAA TAC CGG CCG TGC GTA CTT AGA CA

10 d18S-4238 (SEQ ID NO:18)

AAA AAA AAA AAA AAA TGC CCT CCA ATG GAT CCT CGT TA

d18S-5482 (SEQ ID NO:19)

AAA AAA AAA AAA AAA CTA CGG AAA CCT TGT TAC GAC TT

15

Eukaryotic 28S rRNA bridging oligonucleotides

d28S-11599 (SEO ID NO:20)

AAA AAA AAA AAA AAA AAA GAG CAC TGG GCA GAA ATC ACA TC

20

d28S-7979 (SEQ ID NO:21)

AAA AAA AAA AAA AAA GTT TCT TTT CCT CCG CTG ACT AA

d28S-12533 (SEQ ID NO:22)

- 25 AAA AAA AAA AAA AAA AAA TCC TCA GCC AAG CAC ATA CAC CA
 - 2. Binding Buffer (also referred to as hybridization buffer) 3 M TMAC, 10 mM Tris, (pH 7.0)
- 30
- 3. Bridging Nucleic Acid Mixture

Mixtures of 16S, 23S, 18S, and/or 28S bridging oligonucleotides were used. All oligonucleotides were purchased from IDT and purified from polyacrylamide gels.

- 4. Capture Nucleic Acid (Oligo(dT) MagBeads) Seradyn MGOL #2815-2103.
- 35 5. Wash Solution
 - 2 M TMAC, 6.67 mM Tris (pH 7.0) (this is a dilution of binding buffer).

EXAMPLE 2: Methods for rRNA Depletion from Prokaryotic Total RNA

The following methods are provided by way of example for practicing methods of the invention. They have been performed and shown to effect methods of the invention. The invention is not intended to be limited to these protocols, and it is specifically contemplated that variations of the methods below may be employed that fall within the scope of the invention if they effect depletion, isolation, or separation of a targeted nucleic acid, particularly rRNA.

This example demonstrates the depletion of 16S and 23S rRNA from E. coli total RNA.

10 RNA/Bridging Nucleic Acid Mixture Annealing

RNA (10 μ g/15 μ l) was added to 200 μ l of binding buffer. The bridging nucleic acid mixture consisted of d16S-807 (5 μ M), d16S-1092 (5 μ M), d23S-1954 (5 μ M), d23S-2511 (5 μ M). The bridging nucleic acid mixture (4 μ l) was added to the RNA and the mixture was incubated at 70°C for 10 minutes and then shifted to 37°C for 30 minutes.

Thirty minutes was found to be an adequate time for the annealing step. Longer time periods can be used with no adverse effects. Between fifteen and 120 minutes have been used successfully in the methods of the invention.

Preparation of Capture Nucleic Acid

Capture nucleic acid (Oligo (dT) MagBeads, Seradyn) in storage buffer was mixed and 50 µl was removed to a separate tube. A magnetic stand was applied to the side of the tube to capture the magnetic beads and the supernatant was removed. The capture nucleic acid was equilibrated one time with distilled, deionized water (50 µl) and once with binding buffer (50 µl). The captured nucleic acid was captured again with a magnetic stand, and the binding buffer wash was removed. The magnetic beads were resuspended in 50 µl of binding buffer.

25 rRNA Capture

5

15

20

30

Following the 30 minute annealing of RNA with the bridging nucleic acid mixture, the capture nucleic acid was added and the mixture was incubated at room temperature for 15 minutes. A magnetic stand was then applied to the tube to capture the magnetic beads. The supernatant containing mRNA, 5S rRNA, and tRNAs was removed to another tube and saved. An optional washing step was performed next. The magnetic beads were washed with Wash

Solution (100 μ l) and captured again. The wash supernatant was removed and added to the original supernatant.

Fifteen minutes was found to be an adequate time for rRNA capture. Longer time periods can be used with no adverse effects. rRNA capture likely occurs rapidly, and capture times of 5 minutes – 60 minutes have been used successfully in the methods of the invention.

Precipitating mRNA

5

10

15

20

25

mRNA, 5S rRNA, and tRNAs were precipitated by adding 1/10 volume of 3M NaOAc (pH 5.5) and 3 volumes of 100% EtOH and incubating at -20°C for 60 minutes. The precipitated RNA was pelleted in a microfuge, washed with 70% EtOH, and resuspended in TE (pH 8.0).

Analysis of Purified mRNA

Purified mRNA was analyzed with the Caliper RNA 6000 LabChip kit on an Agilent Bioanalyzer. Purified RNA was compared with a control *E. coli* total RNA sample that was carried through the reaction as described above, except that the Bridging Nucleic Acid Mixture was left out. This assay system uses electrophoretic and electrokinetic separation in a capillary electrophoresis type system. The rRNAs appear as peaks on an electropherogram (FIG. 3). The percentage of a rRNA present in the sample is calculated from the area under the peak.

Under the protocol conditions described above, the 5S + tRNA peak area is essentially the same in the control and in experimental samples. The % of 16S or 23S rRNA removed was calculated using the ratios of $16S_{peak area}/5S_{peak area}$ and $23S_{peak area}/5S_{peak area}$. Enriched and control RNAs with similar 5S + tRNA peak areas were compared.

% 16S rRNA removed =

A corresponding formula was used to calculate % 23S rRNA removed.

Electropherograms of RNA from a control reaction and from an experimental reaction after ribosomal RNA depletion are shown in FIG. 3 and FIG. 4.

EXAMPLE 3:

Evaluations of Efficacy with Prokaryotic Targets

The materials and methods of Examples 1 and 2 were employed to determine the efficiency of removal of 16S rRNA or 23S rRNA or both from *E. coli* total RNA. Changes in the parameters of the experiments are noted when appropriate. These experiments were performed to evaluate the efficacy of various bridging nucleic acids and reaction conditions.

The following results are from reactions that employed 10 µg of *E. coli* total RNA, 40 pmol of total 16S rRNA bridging nucleic acid, 40 pmol of total 23S rRNA bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1.

Bridging Nucleic Acid	% 16S Removed average of 2	% 23S Removed average of 2
16S/23S	reactions	reactions
d16S-358/d23S-2511	96.48285	89.86496
d16S-537/d23S-1954	97.47974	91.32074
d16S-537/d23S-2511	97.48704	91.216
d16S-807/d23S-1954	95.79126	89.85388
d16S-807/d23S-2511	95.25362	91.06399
d16S-1092/d23S-1118	97.91265	96.50658
d16S-1092/d23S-1954	96.7473	89.40605
d16S-1092/d23S-2511	97.61689	91.5964
d16S-358/d23S-1954	96.74434	88.07242
d16S1092/d23S-1954	97.19134	98.44728
(20 pmol)		
d23S-2511 (20 pmol)		

10

5

The following results are from reactions that employed 10 μ g of *E. coli* total RNA, 26 pmol of 16S rRNA bridging nucleic acid, 26 pmol of 23S rRNA bridging nucleic acid, and 35 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092/d23S-1118	97.38534	95.02083
d16S-1092/d23S-1957	97.8291	90.798

The following results are from reactions that employed 10 μ g of E. coli total RNA, 75 pmol of 16S rRNA bridging nucleic acid, 75 pmol of 23S rRNA bridging nucleic acid, and 100 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092d23S-1118	99.14812	99.11895
d16S-1092d23S-1954	98.79938	98.45245
d16S-1092d23S-2511	99.00567	98.84033

The following results are from reactions that employed 10 μ g of *E. coli* total RNA, 37.5 pmol of 16S rRNA bridging nucleic acid, 37.5 pmol of 23S rRNA bridging nucleic acid, and 50 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 168/238	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092/d23S-1118	98.95563	98.28748
d16S-1092/d23S-1954	97.83593	94.84438

10

The following results are from reactions that employed 10 μ g of E. coli total RNA, 75 pmol of 16S rRNA bridging nucleic acid or 75 pmol of 23S rRNA bridging nucleic acid with 75 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed	% 23S Removed
n.a./d23S-581	70 105 Removed	98.98529
n.a./d23S-581	-	98.87251
n.a./d23S-1118	-	93.62175
n.a./d23S-1118	-	91.4927
n.a./d23S-1954	-	98.68262
n.a./d23S-1954	_	99.03237
n.a./d23S-2511	-	99.31982
n.a./d23S-2511	-	99.13291
d16S-358/n.a.	97.65586	-
d16S-358/n.a.	97.51393	
d16S-537/n.a.	99.16427	-
d16S-537/n.a.	98.92345	-
d16S-807/n.a.	98.0661	•
d16S-807/n.a.	98.14292	•

n.a. = not applicable

5

10

The following results are from reactions that employed 5 μ g of *E. coli* total RNA, 25 pmol of each 16S rRNA or 23S rRNA bridging nucleic acid, and 25 μ l of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing reaction was for 60 minutes at 37°C.

63

Bridging Nucleic Acid 16S/23S	% 16S Removed	% 23S Removed
n.a./d23S-488	-	~100
n.a./d23S-1118	-	~100
d16S-3'/d23S-488	89.024	94.228
d16S-548/d23S-488	~100	93.718
d16S-1092/d23S-488	~100	92.652

The following results are from reactions that employed 5 μ g of E. coli total RNA, 16S rRNA bridging nucleic acid as indicated, 23S rRNA bridging nucleic acid as indicated, and 25 μ l of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing reaction was for 120 minutes at 37°C.

Bridging Nucleic Acid 16S/23S	% 16S Removed	% 23S Removed
d16S-3' (25 pmol)/n.a.	89.137	-
d16S-548 (25 pmol)/n.a.	~100	-
d16S-1092 (25 pmol)/n.a.	~100	-
d16S-3' (25 pmol)		
d16S-548 (25 pmol)/n.a.	~100	-
d16S-3' (25 pmol)		
d16S-1092 (25 pmol)/n.a.	~100	-
d16S-548 (25 pmol)		
d16S-1092 (25 pmol)n.a.	~100	-
d16S-548 (25 pmol)/		
d23S-3' (25 pmol)	~100	~100
d16S-1092 (25 pmol)/		
d23S-3' (25 pmol)	~100	~100
d16S-3' (25 pmol)/		
d23S-3' (25 pmol)	92	~100

EXAMPLE 4: The Effect of Washing the Capture Nucleic Acid

The purpose of this experiment was to determine if washing the capture nucleic acid and combining the wash with the purified mRNA had an effect on the presence of rRNA in the purified mRNA sample. Reactions employed 10 μ g of E. coli total RNA, 75 pmol d16S-1092, 75 pmol of d23S-d1118, and 100 μ l of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing reaction proceeded for 60 min at 37°C. After the nucleic acid capture step, the capture nucleic acid (with bound rRNA) was resuspended and washed with 100 μ l of the indicated solution at room temperature for 5 minutes. The capture nucleic acid was re-captured with a magnetic stand and the supernatant was removed and combined with mRNA in the supernatant from the first capture. mRNA in the combined supernatants were precipitated with ethanol and evaluated with RNA 6000 Lab Chip assay for the presence of rRNAs. The percent of rRNA removal for the entire process is indicated in the table below.

Wash	% 16S Removed	% 23S Removed
0.4 M TMAC	66.061	66.175
1.0 M TMAC	95.810	96.708
1.5 M TMAC	~100	~100
2.0 M TMAC	~100	~100

15

25

5

10

These results demonstrate that lowering the molarity of the TMAC wash solution increases the stringency of the rRNA capture reaction when the temperature is held constant at room temperature. The results also demonstrate that washing the capture nucleic acid magnetic beads with 1.5 and 2.0 M TMAC does not remove rRNA from the capture nucleic acid.

20 EXAMPLE 5:

Evaluation of Efficacy with Prokaryotic and Eukaryotic rRNA Targets

The purpose of this example was to evaluate efficacy of the methods of the invention for depleting 16S rRNA, 18S rRNA, 23S rRNA, and 28S rRNA from mixtures of prokaryotic and eukaryotic total RNA. Depletion methods were verified using various mammalian samples, including rat livers.

Equal amounts (2.5 μ g) of *E. coli* total RNA and rat liver total RNA were mixed prior to the mRNA enrichment procedure. The bridging oligonucleotides employed were:

65

	d16S-1092	(10 pmol)
	d16S-807	(10 pmol)
	d23S-1954	(10 pmol)
	d23S-2511	(10 pmol)
5	d18S-3711	(20 pmol)
	d28S-11599	(20 pmol)

The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to methods in Example 2. The results are shown in FIG. 5A and 5B. Note that all rRNAs were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 6:

Evaluation of Efficacy with Human rRNA Targets

Additional experiments were done using human samples to evaluate the extent of human rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from human liver total RNA. rRNAs were depleted from human liver total RNA (5 µg). The bridging oligonucleotides employed were:

d18S-3711 (40 pmol) d28S-11599 (40 pmol)

20

30

10

The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 6A and 6B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

25 EXAMPLE 7:

Evaluation of Efficacy with Rat rRNA Targets

Additional experiments were done using rat samples to evaluate the extent of rat rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from rat liver total RNA. rRNAs were depleted from rat liver total RNA (5 µg). The bridging oligonucleotides employed were:

d18S-3711R-polyA (40 pmol) d28S-11599R-polyA (40 pmol) The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 7A and 7B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

5

10

20

25

EXAMPLE 8:

Evaluation of Efficacy with Mouse rRNA Targets

Additional experiments were done using mouse samples to evaluate the extent of rat rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from mouse liver total RNA (5 μ g). The bridging oligonucleotides employed were:

d18S-3711R-polyA (40 pmol) d28S-11599R-polyA (40 pmol)

The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 8A and 8B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 9:

Use of Purified E. coli mRNA in Gene Array Expression Analysis

mRNA was purified from total E. coli RNA (10 μ g) using the methods of the invention as described in Example 2. A control reaction was also performed in which the bridging nucleic acid mixture was omitted form the reaction. Control total RNA and purified mRNA (1.5 μ g) were added to 70 pmol random hexamers in a final volume of 7.25 μ l. The mixture was heated at 70° C for 10 minutes, then transferred to ice for 3 minutes. The following components were added to each reaction:

 $5 \mu l$ cDNA 1^{st} strand synthesis buffer (Invitrogen)

2.5 µl 0.1 M DTT

1.25 µl 10 mM dATP

67

1.25 μl	10 mM dGTP	
1.25 µl	10 mM dTTP	
5 μΙ	10 mCi/ml	³³ P-dCTP (Perkin Elmer-NEN)
1 μl	Superscript II reverse transcriptase (Invitrogen) 200 U/μl	

5

10

15

The reactions were incubated at 42°C for 120 minutes. Unincorporated nucleotides were removed from the reactions with a Qiaquick PCR cleanup column (Qiagen). The labeled cDNAs (3 x 10⁷ cpm/blot) were used to probe replicate portions of PanoramaTM E. coli gene arrays, using hybridization buffers supplied by the array manufacturer (Sigma-Genosys). The arrays were washed and exposed to film. This example demonstrates a dramatic increase in hybridization signal (sensitivity) on gene arrays when labeled cDNA is prepared from bacterial mRNA, purified according to the methods of the invention, rather than from total RNA.

EXAMPLE 10:

Instructions for Use with Kit

The following instructions have been followed with a kit of the invention described below for the successful depletion of 16S and 23S rRNA from a sample comprising prokaryotic RNA populations. Bridging oligonucleotides with targeting regions complementary to 18S and 28S rRNA may be employed according to the method below to effect a similar result (as in Examples 5-8).

20	Materials Provided with a Kit Embodiment	
	30 <i>μ</i> l	Control RNA
	1.2 ml	Capture Nucleic Acid [as in Example 1]
	7 ml	Binding Buffer [as in Example 1]
	95 μl	Bridging Oligonucleotide Mix [as in Example 2]
25	2.4 ml	Wash Solution [as in Example 1]
	1.75 ml	Nuclease-free Water
	50 ea	RNase-free 1.5 ml tubes
	25 ea	RNase-free 2ml Elution tubes
	200 μl	Glycogen (5 mg/ml)
30	875 μ1	3 M NaOAc

Experimental Parameters

5

10

15

20

25

30

A. RNA Source

This mRNA enrichment procedure is designed to work with purified total RNA from many different bacteria, including both gram-positive and gram-negative species. The procedure was optimized with total *E. coli* RNA and has been found to remove 90-99% of the rRNA from *Bacillus subtilis*, *Staphylococcus aureus*, *Prochlorococcus* sp., *Neisseria meningitidis*, and *Pseudomonas aeruginosa*, for example. It is contemplated that any eubacterial species may be targeted using the methods and compositions of the invention.

This procedure is designed so that small RNAs (including tRNA and 5S rRNA) remain in the enriched mRNA population. However, if the loss of very small RNA species (<200 base) will not be an issue, the initial isolation of total RNA should be performed with Ambion's RNAQUEOUS KIT. The RNAQUEOUS KIT will remove most small RNA species and provide the highest possible level of mRNA enrichment. If small RNAs are of interest to the user, it is best to avoid glass fiber filter-based purification.

B. Precipitate RNA to remove salt and concentrate if necessary

Total RNA prepared from a solid-phase extraction method such as RNAQUEOUS can be used immediately after elution because such samples are unlikely to have high levels of salt. On the other hand, RNA isolated by methods that include organic extractions, for example using the products RNAWIZ, TRIZOL or ToTALLY RNA, may have a substantial amount of residual salt. If RNA from these types of procedures has been precipitated only a single time, we recommend doing a second alcohol precipitation and 70% EtOH wash to remove residual salt before starting the enrichment procedure.

The recommended maximum amount of RNA per reaction is 10 μ g and the recommended maximum volume for the RNA is 15 μ l. If the RNA sample is too dilute, it will be necessary to precipitate and concentrate the RNA to at least 10 μ g/15 μ l. Precipitate the RNA with:

- 0.1 volume 5 M Ammonium Acetate or 3 M sodium acetate
- 1 μl Glycogen (The glycogen acts as a carrier to increase precipitation efficiency from dilute RNA solutions; it is unnecessary for solutions with 200 μg RNA/ml)
- 2.5 volumes 100% ethanol

69

- a. Leave the precipitation mixture at -20° C overnight, or quick-freeze it in either ethanol and dry ice, or in a -70° C freezer for 30 minutes.
 - b. Recover the RNA by centrifugation at 12,000 x g for 30 minutes at 4°C.
- 5 c. Carefully remove and discard the supernatant. The RNA pellet may not adhere tightly to the walls of the tubes, so we suggest removing the supernatant by gentle aspiration with a fine-tipped pipette.
 - d. Centrifuge the tube briefly a second time, and aspirate any additional fluid that collects with a fine-tipped pipette.
- e. Add 1 ml 70% ethanol, and vortex the tube a few times. Repellet the RNA by microcentrifuging, for 10 minutes at 4°C. Remove supernatant carefully as in steps c and d above.

RNA should be dissolved in TE or Ambion's THE RNA STORAGE SOLUTION. It is important to accurately quantitate RNA so as not to overload the system. Ambion recommends using the RiboGreen RNA Quantitation Assay and Kit (Molecular Probes) or a high quality, calibrated spectrophotometer.

C. Save an aliquot of your total RNA

If possible, retain a small aliquot (\sim 1-2 μ g) of the total RNA used for comparison with enriched mRNA by gel electrophoresis after the procedure is finished.

20 Instructions

15

A. Anneal RNA and Bridging Oligonucleotide Mix

1. Add RNA to Binding buffer

Add total RNA (up to 10 μ g total RNA in a maximum volume of 15 μ l) to 200 μ l Binding Buffer in a 1.5 ml tube provided with the kit. Close the tube and tap or vortex gently to mix.

25 2. Add Bridging Oligonucleotide Mix to RNA

Add 4.0 μ l of the Bridging Oligonucleotide Mix to the RNA in Binding Buffer. Close the tube and tap or vortex gently to mix. Pulse in a microcentrifuge very briefly to get mixture to bottom of tube.

5

20

3. Incubate reactions at 70°C for 10 minutes.

Incubating the mixture at 70°C for 10 minutes denatures secondary structures in RNA, including the 16S and 23S rRNAs, allowing for maximal hybridization of the bridging oligonucleotides to the rRNAs.

4. Incubate reactions at 37°C for 1 hour.

Incubating the mixture at 37°C for 1 hour allows for binding of the bridging oligonucleotides to the 16S and 23S rRNA. The Binding Buffer has been optimized to function specifically and efficiently at this temperature.

B. Prepare the Capture Nucleic Acid

During the 1 hour RNA/Bridging Oligonucleotide Mix annealing step, prepare the Capture Nucleic Acid. The Capture Nucleic Acid is in a 1% (10 mg/ml) suspension, vortex the tube briefly before pipetting to be sure they are well suspended.

1. Aliquot the Capture Nucleic Acid

For each 10 μ g reaction remove 50 μ l Capture Oligos to a 1.5 ml tube. Capture Nucleic Acid for up to 10 reactions can be processed in a single 1.5 ml tube.

2. Wash the Capture Nucleic Acid once with water and once with Binding Buffer

- a. Capture the beads (Capture Nucleic Acid) by placing the tube on the Magnetic Stand. Leave the tube on the stand until all of the Capture Nucleic Acid is arranged inside the tube near the magnet. This will take ~3 minutes for microfuge tubes.
 - b. Carefully remove the supernatant by aspiration, leaving the beads in the tube, and discard the supernatant.
 - c. Add Nuclease Free Water to the captured beads at a ratio of 50 μ l/50 μ l beads).
- d. Remove the tube from the Magnetic Stand, resuspend the beads by gently vortexing briefly, recapture the beads with a Magnetic Stand, carefully aspirate the supernatant, leaving the beads in the tube, and discard the supernatant.
 - e. Add Binding Buffer to the captured beads at a ratio of 50 μ 1/50 μ 1 beads).

f. Repeat step d.

10

20

- 3. Resuspend the Capture Nucleic Acid in Binding Buffer
- Add Binding Buffer to the captured beads at a ratio of 50 μ l/50 μ l beads). a.
- b. Remove the tube from the Magnetic Stand, resuspend the beads by gently tapping the tube or very gentle vortexing. 5
 - ¢. Pulse spin in a microcentrifuge to get liquid to the bottom of the tube.

C. Capture the rRNA with Capture Nucleic Acid and Recover the Enriched **mRNA**

1. Add Capture Nucleic Acid (50 µl/rxn) to RNA/Bridging Oligonucleotide Mix and incubate at RT for 15 minutes.

- After the 1 hour incubation at 37°C (Step A.4) remove tubes to room temperature a (RT) and immediately add 50 µl of the washed and equilibrated beads (Capture Nucleic Acid, from Step B.3c) to each purification reaction. Very gently vortex or tap tube to mix briefly and pulse spin in a microcentrifuge to get liquid to the bottom of the tube.
- b. Incubate 15 minutes at RT. During this step the oligonucleotide sequence on the 15 Capture Nucleic Acid anneals to the bridging oligonucleotides. The bridging oligonucleotides remain hybridized to the 16S and 23S rRNAs. The hybridization "sandwich" of bridging oligonucleotide and capture oligonucleotide (via the capture region on the capture oligo and the bridging region on the bridging oligo) is formed at this step.

2. Recover the supernatant containing the enriched mRNA.

- Capture the beads by placing the tube on the Magnetic Stand. Leave the tube on a. the stand until all of the beads are arranged inside the tube near the magnet. This will take ~3 minutes for microfuge tubes. Allow the beads to be completely captured by the magnet for at least 3 minutes.
- 25 b. Remove the supernatant by aspiration, being careful not to dislodge the beads. Put the supernatant into a 2 ml nipple bottom tube on ice and save. Do not be overly concerned if there seems to be beads in the removed supernatant. The excess can be removed at the end of the procedure. The supernatant contains the enriched mRNA sample.

- 3. Wash the Oligo MagBeads with Wash Solution and recover the wash.
- Add Wash Solution to the captured beads at a ratio of 100 μ l Wash Solution/50 μ l a. beads.
- b. Remove the tube from the Magnetic Stand, resuspend the beads by gently vortexing briefly. 5
 - Incubate at RT for 5 minutes. ¢.
 - d. Recapture the beads with the Magnetic Stand as in step C.2a. Allow the beads to be completely captured by the magnet for at least 3 minutes.
- Remove the supernatant by aspiration, being careful not to dislodge the beads. e. 10 Put this supernatant in the 2 ml nipple bottom tube on ice with that from step C.2b.
 - D. Precipitate and resuspend the enriched mRNA in the supernatant.
 - 1. Perform an EtOH precipitation on the collected supernatant.
- Add 1/10 Volume 3M NaOAc (35 µl) and 5 mg/ml glycogen to a final 15 concentration of 100µg/ml (7 µl) to the supernatant from step C.3.e. (the supernatant volume should be $\sim 350 \mu l$).
 - b. Briefly vortex the sample to mix.
 - Add 3 Vol. ice cold 100% EtOH (1175 μ l) and mix well by vortexing the sample. c.
 - d. Precipitate the sample at -20°C for at least 1 hour.
- 20 Centrifuge the sample for 30 min. @ 13,000 rpm. e.
 - f. Carefully decant the supernatant.
 - Add 750ml ice cold 70% EtOH, vortex briefly, and centrifuge for 5 min. @ g. 13,000 rpm. Decant the supernatant.
 - h. Repeat step D.1.g.

15

20

25

30

73

i. After decanting the supernatant spin briefly to collect. Remove the remaining supernatant with a pipettor, being careful not to dislodge the pellet. Air dry for 5 min.

2. Resuspend the enriched mRNA in an appropriate buffer.

- a. After the pellet has air dried for no more than 5 min. add 2 μl TE pH 8.0 (RNA
 5 STORAGE SOLUTION, 1 mM EDTA or Nuclease-Free ddH₂O could be substituted).
 - b. Allow the RNA to resuspend for 15 min. at room temperature. Vortex the sample vigorously to resuspend. Collect the sample by brief centrifugation. NOTE: If the pellet refuses to go into solution the sample can be incubated for 5 min. @ 70°C. This should help resuspend the pellet. NOTE: Often there will be beads remaining in the sample after the precipitation (This will cause the RNA solution to appear brownish in color). This can be remedied by applying the sample to the Magnetic stand for ~3 min. and removing the supernatant to a new tube.

E. Compatibility with respect to other microorganisms

Based on experimental evidence and sequence information, the following organisms should be compatible (removal of 16S rRNA and of 23S rRNA) with the oligos identified in Example 1 (non-control oligos): Acidithiobacillus ferrooxidans, Acinetobacter calcoaceticus, Actinobacillus actinomycetemcomitans, Aeromonas hydrophila, Agrobacterium tumefaciens, Alcaligenes faecalis, Bacillus alcalophilus, Bacillus anthracis, Bacillus cereus, Bacillus halodurans, Bacillus licheniformis, Bacillus mycoides, Bacillus subtilis, Bacillus thuringiensis, Bartonella bacilliformis, Bordetella avium, Bordetella bronchiseptica, Bordetella parapertussis, Bordetella pertussis, Borrelia burgdorferi, Bradyrhizobium japonicum, Bradyrhizobium lupini, Brevundimonas diminunata, Brucella melitensis, Brucella melitensis biovar suis, Buchnera aphidicola, Buchnera sp. APS, Burkholderia cepacia, Burkholderia mallei, Burkholderia pseudomallei, Caulobacter crescentus, Chlamydia muridarum, Chlamydia suis, Chlamydia Chlamydophila Chlamydophila felis, trachomatis. Chlamydophila abortus, caviae, Chlamydophila pecorum, Chlamydophila pneumoniae, Chlamydophila psittaci, Chlorobium limicola, Chlorobium tepidum, Citrobacter freundii, Clostridium acetobutylicum, Clostridium difficile, Clostridium histolyticum, Corynebacterium diptheriae, Corynebacterium glutamicum, Cytophaga hutchisonii, Desulfovibrio vulgaris, Dichelobacter nodosus, Enterococcus asini, Enterococcus avium, Enterococcus casseliflavus, Enterococcus cecorum, Enterococcus

10

15

20

25

30

PCT/US02/41014

columbae, Enterococcus dispar, Enterococcus durans, Enterococcus faecalis, Enterococcus faecium, Enterococcus flavescens, Enterococcus gallinarum, Enterococcus hirae, Enterococcus malodoratus, Enterococcus mundtii, Enterococcus pseudoavium, Enterococcus raffinosus, Enterococcus saccharolyticus, Enterococcus sulfureus, Erwinia chrysanthemi, Escherichia coli, Fibrobacter succinogenes, Frankia Sp., Fusobacterium nucleatum, Geobacillus Geobacter sulfurreducens, Gluconacetobacter europaeus, stearothermophilus. Gluconacetobacter intermedius, Gluconacetobacter xylinus, Haemophilus ducreyi, Haemophilus influenzae, Klebsiella pneumoniae, Lactobacillus amylolyticus, Lactobacillus delbrueckii, Lactococcus lactis, Leuconostoc carnosum, Leuconostoc lactis, Leuconostoc mesenteroides, Listeria gravi, Listeria innocua, Listeria ivanovii, Listeria monocytogenes, Listeria seeligeri, Melissococcus plutonius, Micrococcus luteus, Mycobacterium avium, Mycobacterium avium supsp. Paratuberculosis, Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Myxococcus xanthus, Neisseria gonorrhoeae, Neisseria meningitidis, Nitrosomonas europaea, Pasteurella multocida, Peptococcus niger, Plesiomonas shigelloides, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Ralstonia pickettii, Ralstonia solanacearum, Renibacterium salmoninarum, Rhizobium vitis, Rhodococcus erythropolis, Rhodococcus fascians. Rhodopseudomonas palustris, Rhodospirillum rubrum, Rickettsia akari, Rickettsia australis, Rickettsia bellii, Rickettsia canadensis, Rickettsia conorii, Rickettsia montanensis, Rickettsia parkeri, Rickettsia prowazekii, Rickettsia rhipicephali, Rickettsia rickettsii, Rickettsia sibirica, Rickettsia typhi, Salmonella bongori, Salmonella enterica, Salmonella enteritidis, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shewanella putrefaciens, Sinorhizobium meliloti, Sporosarcina globispora, Staphylococcus aureus, Staphylococcus carnosus, Staphylococcus condimenti, Staphylococcus epidermidis, Stigmatella aurantiaca, Streptococcus equii, Streptococcus gordonii, Streptococcus macedonicus, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus parauberis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus uberis, Streptomyces ambofaciens, Streptomyces coelicolor, Streptomyces griseus, Streptomyces lividans, Streptomyces nodosus, Streptomyces rimosus, Thermoanaerobacter tengcongensis, Thermobifida fusca, Thermomonospora chromogena, Thiobacillus ferrooxidans, Trteponema denticola, Treponema pallidum, Vibrio cholerae, Yersinia enterocolitica, Yersinia pestis, Xanthomonas campestris, Xanthomonas axonopodis pv. Citri, and Xylella fastidiosa.

10

15

20

25

Based on experimental evidence and sequence information, the following organisms should be partially compatible (removal of 23S rRNA and of 50-100% 16S rRNA) with oligos identified in Example 1 (non-control): Azotobacter vinelandii, Bacteroides fragilis, Carboxydothermus hydrogenoformans, Clostridium tyrobutyricum, Desulfovibrio vulgaris, Dictyoglomus thermophilum, Enterococcus solitarius, Erysipelothrix rhusiopathiae, Erysipelothrix tonsillarum, Flexibacter flexilis, Legionella pneumophila, Leptospira interrogans, Leucothrix mucor, Listeria welshimeri, Methylococcus capsulatus, Myroides odoratus, Oenococcus oeni, Paracoccus denitrificans, Pectinatus frisingensis, Porphyromonas gingivalis, Prevotella intermedia, Silicibacter pomeroyi, Tannerella forsythensis, Tetragenococcus halophilus, Thermobispora bispora, Thermus thermophilus and, Thiomonas cuprina

Based on experimental evidence and sequence information, the following organisms should be partially compatible (removal of 16S rRNA and 50-100% of 23S rRNA) with oligos identified in Example 1 (non-control): Burkholderia fungorum, Clostridium perfringens, Desulfitobacterium hafniense, Magnetospirillum magnetotacticum, Mesorhizobium loti, Nannocystis exedens, Novosphingobium aromaticivorans, Parachlamydia acanthamoebae, Ruminobacter amylophilus, Ruminococcus albus, Tropheryma whipplei, and Wolbachia endosymbiont of Drosophila melanogaster.

Based on experimental data and sequence information, the following organisms are believed to be incompatible with the oligos in Example 1: Archaebacteria, Campylobacter spp., Chloroflexus aurantiacus, Cyanobacteria, Dehalococcoides ethenogenes, Deinococcus radiodurans, Fervidobacterium islandicum, Helicobacter pylori, Mycoplasma spp., Pirellula marina, Propionibacterium freundenreichii, Simkania negevensis, Thermotoga maritima, and Ureaplasma urealyticum.

EXAMPLE 11: Methods for Eukaryotic rRNA Depletion from Mixed Human/E. coli Total RNA

These experiments were performed to demonstrate the depletion of 18S and 28S rRNAs from a mixture of prokaryotic and eukaryotic total RNA. Materials from Example 1 were used in the following experiments, except where noted.

30 RNA/Bridging Nucleic Acid Mixture Annealing

RNA (50 µg human total / 0.5 µg E. coli total) in 30 µl TE pH8.0 was added to 300 µl of binding buffer (The binding buffer in this example contains 0.02% Triton-X 100 which has been shown to reduce non-specific interactions between nucleic acids target and the capture nucleic acid). The bridging nucleic acid mixture consisted of d18S-3711, d18S-4238, d18S-5482, d28S-7979, d28S-11599 and d28S-12533 (each of these bridging oligonucleotides is at a final concentration of 3.33 µM). The bridging nucleic acid mixture (20 µl) was added to the RNA and the mixture was incubated at 70° C for 10 minutes and then shifted to 37° C for 1 hour.

Preparation of Capture Nucleic Acid

Capture nucleic acid (Oligo (dT) MagBeads, Seradyn) in storage buffer was mixed and 250 µl was removed to a separate tube. A magnetic stand was applied to the side of the tube to capture the magnetic beads and the supernatant was removed. The capture nucleic acid was equilibrated one time with distilled, deionized water (250 µl) and once with binding buffer (250 µl). The captured nucleic acid was captured again with a magnetic stand, and the binding buffer wash was removed. The magnetic beads were stored on ice until used in the next step (rRNA Capture).

rRNA Capture

5

10

15

20

25

30

Following the 1 hour annealing of RNA with the bridging nucleic acid mixture, the RNA-bridging oligonucleotide mixture was added to the capture nucleic acid (see Preparation of Capture Nucleic Acid), and the mixture was incubated at 37° C for 15 minutes. A magnetic stand was applied to the tube to capture the magnetic beads. The supernatant containing *E. coli* total RNA was removed to another tube and saved. An optional washing step was performed. The magnetic beads were washed with Wash Solution (100 μ l) and captured again. The wash supernatant was removed and added to the original supernatant.

Fifteen minutes was found to be an adequate length of time for rRNA capture. Longer time periods can be used with no adverse effects. rRNA capture occurs rapidly, and capture times of 5-60 minutes have been used successfully in the methods of the invention.

Precipitating RNA

E. coli total RNA was precipitated by adding 1/10 volume of 3 M NaOAc (pH 5.5) and 3 volumes of 100% EtOH and incubating at -20°C for 60 minutes. The precipitated RNA was pelleted in a microfuge, washed with 70% EtOH, and resuspended in TE (pH 8.0).

WO 03/054162

Analysis of Purified RNA

Purified RNA was analyzed with the Caliper RNA 6000 LabChip kit on an Agilent Bioanalyzer. Purified RNA was compared with a control total RNA sample that was carried through the reaction as described above, except that the Bridging Nucleic Acid Mixture was omitted (FIG. 9). The percentage of a rRNA present in the sample is calculated from the area under the peak. The percentage removal of the 18S and 28S rRNAs was calculated as described in Example 2 for removal of 16S and 23S rRNAs.

77

PCT/US02/41014

Electropherograms of RNA from a control reaction and from an experimental reaction after ribosomal RNA depletion are shown in FIG. 9.

10

15

20

25

5

EXAMPLE 12:

Evaluation of efficacy with Mixed Prokaryotic and Eukaryotic rRNA Targets

The purpose of this experiment was to determine if one could, first remove the eukaryotic RNA and subsequently remove the prokaryotic rRNAs from a mixture of the two total RNAs. The materials and methods of Examples 1, 2 and 11 were used, except where noted. Depletion methods were verified using various mammalian samples, including rat liver total RNA, and both *E. coli* and *Bacillus subtilis* total RNA.

25 μ g rat liver total RNA and 2 μ g *E. coli* total RNA were mixed prior to the RNA enrichment procedure. The bridging oligonucleotides employed were: d16S-807, d16S-1092, d23S-1954, d23S-2511, d18S-3711, d18S-4238, d18S-5482, d28S-7979, d28S-11599, and d28S-12533.

The reactions began with procedures similar to Example 11, except for the following changes. 10 µl bridging nucleic acid mixture and 125 µl capture nucleic acid were used to remove the mammalian 18S and 28S rRNAs. Following the wash step, the wash solution and the unbound fraction containing the bacterial RNA were combined. The precipitation step was not performed. Instead the bacterial 16S and 23S rRNA was removed as in Example 2, with the following modifications. The bridging nucleic acid mixture (4 µl) containing d16S-807, d16S-1092, d23S-1954, and d23S-2511 was added directly to the combined wash and unbound

10

15

WO 03/054162 PCT/US02/41014

fractions containing the bacterial RNA. The remainder of the procedure for 16S and 23S rRNA followed the methods from Example 2.

78

Electropherograms of RNA from a control reaction with no bridging nucleic acids (FIG. 10A), from a reaction following 18S and 28S rRNA removal (FIG. 10B), and from a reaction following subsequent removal of 16S and 23S rRNA (FIG. 10C) are shown in FIG. 10.

EXAMPLE 13: <u>Use of Purified E. coli mRNA from Mixed Eukaryotic/Prokaryotic Samples in Gene Array</u> <u>Expression Analysis</u>

mRNA was purified from total *E. coli* RNA (2 μg) in a background of human total RNA (25 μg) using the methods of the invention as described in Example 12 (16S, 23S, 18S and 28S rRNAs were all depleted from the sample). A control reaction was also performed in which the bridging nucleic acid mixtures were omitted from the reaction. Control total RNA (8.4 μg) and purified mRNA (1.0 μg) were added to 160 pmol random decamers in a final volume of 24.5 μl. These RNA amounts represent equal fractions of the control and purified RNA samples after the procedure is complete. The mixture was heated at 70°C for 10 minutes, then transferred to ice for 3 minutes. The following components were added to each reaction:

	12 μl .	cDNA 1 st strand synthesis buffer (Invitrogen)
	6 µl	0.1 M DTT
	3 μl	10 mM dATP
20	3 μl	10 mM dGTP
	3 µl	10 mM dTTP
	5 µl	10 mCi/ml ³³ P-dCTP (Perkin Elmer-NEN)
	1 µl	RNase Inhibitor (cloned)

- 25 The reaction was then incubated at room temperature for 10 minutes, and the following component was added:
 - $2 \mu l$ Superscript II reverse transcriptase (Invitrogen) 200 U/ μl

PCT/US02/41014

The reactions were incubated at 42°C for 120 minutes. Unincorporated nucleotides were removed from the reactions with a Qiaquick PCRTM cleanup column (Qiagen). RNA present in the cDNA probes was hydrolyzed by incubation at 65°C for 10 minutes in .05 N NaOH. The probes were subsequently neutralized with 0.05 M HCl. The labeled cDNAs (5 x 10⁷ cpm/blot) were used to probe replicate portions of PanoramaTM E. coli gene arrays, using hybridization buffers supplied by the array manufacturer (Sigma-Genosys). The arrays were washed and exposed to film. This example demonstrates a dramatic increase in hybridization signal (sensitivity) on gene arrays when labeled cDNA is prepared from enriched bacterial mRNA, purified according to the methods of the invention, rather than from the mixed prokaryotic and eukaryotic total RNA.

Example 14:

Evaluations of Efficacy with non-E.coli Prokaryotic Targets

The materials and methods of Examples 1 and 2 were employed in the Examples below except where noted. These experiments were performed to evaluate the efficacy of various bridging nucleic acids with different bacterial species.

Additional targeting regions for prokaryotic 16S and 23S rRNAs were designed. The targeting regions are shown, in the examples below, 3' of the bridging regions. Thus, the targeting region encompasses the remaining, non-bridging region of each molecule described below. SEQ ID NOs are provided for the targeting regions of the bridging nucleic acids provided below (i.e., sequence of bridging regions not included in SEQ ID NO.). Furthermore, the oligos have been further designated with a suffix at the end of the oligo number. CY refers to cyanobacteria; P refers to pseudomonas; R refers to rhodobacter; and CH refers to campylobacter/helicobacter.

16S prokaryotic rRNA bridging oligonucleotides

25 d16S-1114P

5

10

15

20

5'-AAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTACGGGACTT-3' (SEQ ID NO:74)

d16S-1114R

5'-AAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTGCCGGACTT-3' (SEQ ID NO:75)

30 d16S-364

5'-AAAAAAAAAAAAAAAAAAAATCCCCACTGCTGCCTCCCGTAGG-3' (SEQ ID NO:76)

d16S-1087

80

5'-AAAAAAAAAAAAAAAAAAATCCCCACTGCTGCCTCCCGTAGG-3' (SEQ ID NO:78)

5 d16S-534CY

5'-AAAAAAAAAAAAAAAAAAATTACCGCGGCTGCTGGCACGGA-3' (SEQ ID NO:79)

d16S-928CY

10 5'-AAAAAAAAAAAAAAAAACCCCGTCAATTCCTTTGAGTTTC-3' (SEQ ID NO:80)

d16S-1087CY

15

23S prokaryotic rRNA bridging oligonucleotides

d23S-479RCH

5'-AAAAAAAAAAAAAAAATTTCACCTTTCCCTCACGGTACT-3' (SEQ ID NO:82)

20 d23S-485

5'-AAAAAAAAAAAAAAAAAAAAAGGTTCTTTTCACCTTTCCCTCGC-3' (SEQ ID NO:83)

d23S-518 CH

5'-AAAAAAAAAAAAAAAAAAATGGTTTCAGGTTCTATTTCACTC-3' (SEQ ID NO:84)

25

d23S-1954 CH

5'-AAAAAAAAAAAAAAAATTTAACCGACAAGGAATTTCGC-3' (SEQ ID NO:85)

d23S-485CY

30 5'-AAAAAAAAAAAAAAAAAGGTTCTTTTCACCTTTCCCTCGC-3' (SEQ ID NO:86)

The following results are from reactions that employed 10 µg of *Pseudomonas* aeruginosa total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	90.1%	99.5%
d16S-807 (20 pmol), d16S-1114P (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	97.0%	99.4%

81

The following results are from reactions that employed 10 μ g of *Bacillus subtilis* total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	98.2%	96.2%

5

The following results are from reactions that employed 10 µg of *Campylobacter fetus* total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1. The *Campylobacter fetus* 23S rRNA is processed into two fragments (FIG. 12).

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S (1260 nt fragment) Removed average of 2 reactions	% 23S (1667 nt fragment) Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	97.3%	97.7%	89.3%
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-518CH (20 pmol), d23S-2511 (20 pmol)	95.9%	96.7%	89.5%

10

15

The following results are from reactions that employed 10 μ g of *Rhodobacter sphaeroides* total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 μ l of capture nucleic acid, as described in Example 1 unless otherwise noted . The *Rhodobacter sphaeroides* 23S rRNA is processed into two fragments (FIG. 13). One fragment migrates with the 16S rRNA.

Bridging Nucleic Acid 168/238	% 16S + 23S fragment (1600 nt) Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	81.6%	96.8%
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-518CH (20 pmol), d23S-2511 (20 pmol)	95.9%	96.7%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	89.3%	96.1%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol), d23S-2511 (20 pmol)	97.0%	96.4%
d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	83.1%	96.5%
d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	90.2%	95.9%
d16S-537 (20 pmol), d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	96.7%	94.9%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	95.0%	90.2%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	95.2%	89.8%

The results demonstrate that bridging nucleic acids will function with various species. This example also demonstrates functionality based on sequence comparison, *i.e.*, that a bridging oligonucleotide will function with rRNAs in different organisms based on sequence identity between the oligonucleotide and the rRNA of the organism.

Evaluations of Efficacy with Cyanobacteria Targets

5

The materials and methods of Examples 1 and 2 were employed except where noted. These experiments were performed to evaluate the efficacy of various bridging nucleic acids with different bacterial species.

The following results are from reactions that employed 10 µg of Anabaena spp. total RNA, the indicated amounts of the bridging nucleic acids, and 50 µl of capture nucleic acid described in Example 1. The Anabaena sp. 23S rRNA and 23S rRNAs from other cyanobacteria may be processed into several fragments (FIG. 14).

Bridging Nucleic Acid 168/23S		% 16S Removed	l	23S Remov age of 2 reac	
168/23S 2 reactions fragment fragment fragment d16S-364CY (20 pmol), d16S-928CY (20 pmol), d16S-1087CY (20 pmol), d16S-1087CY (20 pmol), d16S-1087CY (20 pmol), d16S-1087CY (20 pmol), d16S-193.7 NA NA NA d16S-364CY (20 pmol), d23S-1954 (Bridging Nucleic Acid				
d16S-364CY (20 pmol) d16S-928CY (20 pmol) d16S-928CY (20 pmol) d16S-928CY (20 pmol) d16S-1087CY (20 pmol) d16S-1087CY (20 pmol) d16S-1087CY (20 pmol) d23S-485 (20 pmol) d23S-1094		, –			ľ
928CY (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 99.5 99.5 96.5 84.7 97.7 99.5 11 (20 pmol) d16S-364CY (20 pmol), d16S- 99.2 99.2 96.3 87.2 98.5 98.5 98.7 98.6 88.3 98.3 98.3 1087CY (20 pmol) d16S-364CY (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d33S-485 (20 pmol), d23S- 1954 (20 pmol) d33S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d23S- 1954 (20 pmol), d23S- 1954 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d23S- 1954 (30 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S-	d16S-364CV (20 pmol) d16S-				
d163-364CY (20 pmol), d16S- 93.1	· - '	74.5	1471	1412	****
1087CY (20 pmol)	d16S-364CV (20 pmol) d16S-	93.1	NΔ	NA	NA
d16S-928CY (20 pmol) 93.7 NA NA NA 1087CY (20 pmol) 423S-485 (20 pmol), d23S- NA 98.6 94.1 99.5 1954 (20 pmol) 423S-485 (20 pmol), d23S- NA 98.4 95.7 99.5 2511 (20 pmol) 416S-364CY (20 pmol), d16S- 99.5 96.5 84.7 97.7 416S-364CY (20 pmol) 423S-485, (20 pmol), d23S- 99.2 96.3 87.2 98.5 928CY (20 pmol) 423S-485, (20 pmol), d23S- 99.2 96.3 87.2 98.5 928CY (20 pmol) 423S-485, (20 pmol), d23S- 2511 (20 pmol) 2511 (20 pmol) 88.3 98.3 928CY (20 pmol) 423S-485 (20 pmol), d23S- 99.7 99.2 89.2 99.1 46S-364CY (20 pmol) 423S-485 (20 pmol), d23S- 99.7 99.2 89.2 99.1 416S-364CY (20 pmol) 423S-485 (20 pmol), d23S- 96.9 97.6 86.8 98.9 416S-292CY (20 pmol) 416S-20 pmol) 423S-485 (20 pmol) 423S-485 (20 pmol) 423S-485 (20 pmol) 416S-36	1087CY (20 pmol)	75.1	1421	1412	1 111
1087CY (20 pmol) 23S- 108	d16S-928CV (20 pmol) d16S-	93.7	NA	NA	NA
d23S-485 (20 pmol), d23S-1954 (20 pmol), d23S-1954 (20 pmol) d16S-364 (20 pmol), d23S-1954 (20 pmol), d23S-1954 (20 pmol) d16S-364 (20 pmol), d23S-1954 (20 pmol) d16S-364 (20 pmol), d23S-1954 (20 pmol) d23S-1954 (25 pmol) d23S-195)5.7	1111	1111	'''
1954 (20 pmol)	d23S-485 (20 pmol), d23S-	NA	98.6	94.1	99.5
d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S- 299.5 96.5 84.7 97.7 d16S-364CY (20 pmol), d16S- 299.5 96.5 84.7 97.7 d23S-485, (20 pmol), d23S- 1954 (20 pmol), d16S- 299.2 96.3 87.2 98.5 d16S-364CY (20 pmol), d23S- 2511 (20 pmol) d23S-485, (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S- 485 (20 pmol), d23S- 2511 (20 pmol) d23S- 485 (20 pmol), d23S- 1954 (20 pmol) d23S- 485 (20 pmol), d23S- 1954 (20 pmol) d23S- 485 (20 pmol), d23S- 1954 (25 pmol) d23S- 485 (20 pmol), d23S- 1954 (30 pmol) d23S- 485 (20 pmol), d23S- 1954 (30 pmol) d23S- 485 (20 pmol),	1954 (20 pmol)	1 111	70.0	,	77.5
2511 (20 pmol) d16S-364CY (20 pmol), d16S- 99.5 98.5 98.7 99.5 98.7 99.7 98.6 99.2 98.7 98.6 99.2 98.7 98.7 98.7 98.7 98.7 98.7 98.7 98.7	d23S-485 (20 pmol), d23S-	NA	98.4	95.7	99.5
d16S-364CY (20 pmol), d16S- 928CY (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 928CY (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d16S-364CY (20 pmol), d16S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 1987CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 1087CY (20 pmol) d16S-364 (17.5 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol)	2511 (20 pmol)				1
928CY (20 pmol) d23S-485, (20 pmol), d16S- 99.2 96.3 87.2 98.5 928CY (20 pmol) d23S-485, (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (17.5 pmol), d23S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol)	d16S-364CY (20 pmol), d16S-	99.5	96.5	84.7	97.7
d23S-485, (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 99.2 96.3 87.2 98.5 928CY (20 pmol) d23S-485, (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (15 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S-					
1954 (20 pmol) 168-364CY (20 pmol), d168-99.2 96.3 87.2 98.5	d23S-485, (20 pmol), d23S-				
d16S-364CY (20 pmol), d16S-928CY (20 pmol), d23S-2511 (20 pmol) d23S-485, (20 pmol), d23S-1087CY (20 pmol), d23S-1954 (20 pmol), d23S-1087CY (20 pmol), d23S-2511 (20 pmol) d23S-485 (20 pmol), d23S-1087CY (20 pmol) d23S-485 (20 pmol), d23S-1087CY (20 pmol) d23S-485 (20 pmol), d23S-1954 (20 pmol) d23S-485 (20 pmol), d23S-1954 (20 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) d23S-485 (20 pmol), d23S-1954 (30 pmol)					<u> </u>
928CY (20 pmol) d23S-485, (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-	d16S-364CY (20 pmol), d16S-	99.2	96.3	87.2	98.5
2511 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol)					
d16S-364CY (20 pmol), d16S-1087CY (20 pmol), d23S-1954 (25 pmol), d23S-1087CY (15 pmol) d23S-1087CY	d23S-485, (20 pmol), d23S-				
1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S-	2511 (20 pmol)				
d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (12.5 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1954 (20 pmol), d23S-	d16S-364CY (20 pmol), d16S-	99.7	98.6	88.3	98.3
1954 (20 pmol) d16S-364CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d16S-364 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d16S-364 (15 pmol), d23S- 1954 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
d16S-364CY (20 pmol), d16S-1087CY (20 pmol) 99.7 99.2 89.2 99.1 1087CY (20 pmol) d23S-485 (20 pmol), d23S-2511 (20 pmol) 96.9 97.6 86.8 98.9 1087CY (20 pmol) d23S-485 (20 pmol), d23S-2511 (20 pmol) 99.2 98.2 88.1 97.7 1087CY (20 pmol) d23S-485 (20 pmol), d23S-1954 (20 pmol) 99.8 98.8 88.4 98.5 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) 99.8 99.1 90.6 98.3 1087CY (15 pmol) d23S-485 (20 pmol), d23S-1954 (30 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-1954 (30 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) 99.9 98.7 92.5 98.9	d23S-485 (20 pmol), d23S-				
1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol)					
d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1087CY (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		99.7	99.2	89.2	99.1
2511 (20 pmol) d16S-928CY (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol)					
d16S-928CY (20 pmol), d16S-1087CY (20 pmol) 96.9 97.6 86.8 98.9 1087CY (20 pmol) d23S-485 (20 pmol), d23S-2511 (20 pmol) 99.2 98.2 88.1 97.7 1087CY (20 pmol) d23S-485 (20 pmol), d23S-1954 (20 pmol) 99.8 98.8 88.4 98.5 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) 99.8 99.1 90.6 98.3 1087CY (15 pmol) d23S-485 (20 pmol), d23S-1954 (30 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) 423S-485 (20 pmol), d16S-1087CY (12.5 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) 423S-485 (20 pmol), d23S-1087CY (12.5 pmol) 423S-485 (20 pmol), d23S-1087CY (12.5 pmol) 99.9 98.7 92.5 98.9					
1087CY (20 pmol) d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-	2511 (20 pmol)				
d23S-485 (20 pmol), d23S- 2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		96.9	97.6	86.8	98.9
2511 (20 pmol) d16S-364 (20 pmol), d16S- 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d16S-364 (12.5 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
d16S-364 (20 pmol), d16S- 99.2 98.2 88.1 97.7 1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) 416S-364 (17.5 pmol), d16S- 99.8 98.8 88.4 98.5 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) 99.8 99.1 90.6 98.3 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S- 99.9 98.7 92.5 98.9 d23S-485 (20 pmol), d23S- d23S-485 (20 pmol), d23S- 99.9 98.7 92.5 98.9					
1087CY (20 pmol) d23S-485 (20 pmol), d23S- 1954 (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d16S-364 (12.5 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S-	2511 (20 pmol)		20.0	00.1	07.7
d23S-485 (20 pmol), d23S- 1954 (20 pmol), d16S- 1087CY (17.5 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d16S-364 (12.5 pmol) d16S-364 (12.5 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		99.2	98.2	88.1	97.7
1954 (20 pmol) d16S-364 (17.5 pmol), d16S- 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol) d16S-364 (12.5 pmol) d23S-485 (20 pmol), d23S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
d16S-364 (17.5 pmol), d16S-1087CY (17.5 pmol) 99.8 98.8 88.4 98.5 1087CY (17.5 pmol) d23S-485 (20 pmol), d23S-1954 (25 pmol) 99.8 99.1 90.6 98.3 1087CY (15 pmol) 423S-485 (20 pmol), d23S-1954 (30 pmol) 99.9 98.7 92.5 98.9 1087CY (12.5 pmol) 423S-485 (20 pmol), d23S-1087CY (12.5 pmol) 99.9 98.7 92.5 98.9					
1087CY (17.5 pmol) d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		00.9	00 0	00 4	00.5
d23S-485 (20 pmol), d23S- 1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		33. 8	96.8	00.4	98.3
1954 (25 pmol) d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		į			
d16S-364 (15 pmol), d16S- 1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
1087CY (15 pmol) d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-	d169-364 (15 pmol) d169-	909	00 1	90.6	08.3
d23S-485 (20 pmol), d23S- 1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		22.0	22.1	50.0	30.3
1954 (30 pmol) d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
d16S-364 (12.5 pmol), d16S- 1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-					
1087CY (12.5 pmol) d23S-485 (20 pmol), d23S-		99.0	98.7	92.5	98.9
d23S-485 (20 pmol), d23S-		77.7	70.7	12.3	70.7
	1954 (35 pmol)				

84

	% 16S Removed	3	23S Removage of 2 reac	
Bridging Nucleic Acid 16S/23S	average of 2 reactions	520 nt fragment	2090 nt fragment	2470 nt fragment
d16S-364 (10 pmol), d16S- 1087CY (10 pmol) d23S-485 (20 pmol), d23S- 1954 (40 pmol)	99.9	98.9	90.6	98.6

* * * * * * * * *

5

10

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

- 5 U.S. Application No. 09/854,412
 - U.S. Patent No. 4,486,539
 - U.S. Patent No. 4,563,419
 - U.S. Patent No. 4,659,774
- 10 U.S. Patent No. 4,682,195
 - U.S. Patent No. 4,683,202
 - U.S. Patent No. 4,751,177
 - U.S. Patent No. 4,816,571
 - U.S. Patent No. 4,868,105
- 15 U.S. Patent No. 4,894,325
 - U.S. Patent No. 4,959,463
 - U.S. Patent No. 5,124,246
 - U.S. Patent No. 5,141,813
 - U.S. Patent No. 5,200,314
- 20 U.S. Patent No. 5,214,136
 - U.S. Patent No. 5,216,141
 - U.S. Patent No. 5,223,618
 - U.S. Patent No. 5,264,566
 - U.S. Patent No. 5,273,882
- 25 U.S. Patent No. 5,288,609
 - U.S. Patent No. 5,378,825
 - U.S. Patent No. 5,412,087
 - U.S. Patent No. 5,428,148
 - U.S. Patent No. 5,432,272
- 30 U.S. Patent No. 5,445,934
 - U.S. Patent No. 5,446,137
 - U.S. Patent No. 5,457,025

- U.S. Patent No. 5,466,786
- U.S. Patent No. 5,470,967
- U.S. Patent No. 5,500,356
- U.S. Patent No. 5,539,082
- 5 U.S. Patent No. 5,554,744
 - U.S. Patent No. 5,574,146
 - U.S. Patent No. 5,589,335
 - U.S. Patent No. 5,602,240
 - U.S. Patent No. 5,602,244
- 10 U.S. Patent No. 5,610,289
 - U.S. Patent No. 5,614,617
 - U.S. Patent No. 5,623,070
 - U.S. Patent No. 5,645,897
 - U.S. Patent No. 5,652,099
- 15 U.S. Patent No. 5,670,663
 - U.S. Patent No. 5,672,697
 - U.S. Patent No. 5,681,947
 - U.S. Patent No. 5,700,922
 - U.S. Patent No. 5,702,896
- 20 U.S. Patent No. 5,708,154
 - U.S. Patent No. 5,709,629
 - U.S. Patent No. 5,714,324
 - U.S. Patent No. 5,714,331
 - U.S. Patent No. 5,714,606
- 25 U.S. Patent No. 5,719,262
 - U.S. Patent No. 5,723,597
 - U.S. Patent No. 5,736,336
 - U.S. Patent No. 5,744,305
 - U.S. Patent No. 5,759,777
- 30 U.S. Patent No. 5,763,167
 - U.S. Patent No. 5,766,855
 - U.S. Patent No. 5,773,571

- U.S. Patent No. 5,777,092
- U.S. Patent No. 5,786,461
- U.S. Patent No. 5,792,847
- U.S. Patent No. 5,858,988
- U.S. Patent No. 5,859,221 5
 - U.S. Patent No. 5,872,232
 - U.S. Patent No. 5,886,165
 - U.S. Patent No. 5,891,625
 - U.S. Patent No. 5,897,783
- 10 U.S. Patent No. 5,908,845
 - U.S. Patent No. 5,945,525
 - U.S. Patent No. 6,001,983
 - U.S. Patent No. 6,013,440
 - U.S. Patent No. 6,037,120
- 15 U.S. Patent No. 6,060,246
 - U.S. Patent No. 6,090,548
 - U.S. Patent No. 6,110,678
 - U.S. Patent No. 6,140,496
 - U.S. Patent No. 6,203,978
- 20 U.S. Patent No. 6,221,581
 - U.S. Patent No. 6,228,580
 - U.S. Patent No. 6,309,823
 - U.S. Patent No. 6,316,193
 - U.S. Patent No. 6,322,971
- 25 U.S. Patent No. 6,324,479
 - U.S. Patent No. 6,329,140
 - U.S. Patent No. 6,329,209
- EP 266,032 30
 - PCT/EP/01219
 - PCT/US00/29865

WO 01/32672

WO 86/05815

WO90/06045

WO 92/20702

5

The entire issue of Current Opinion in Microbiology, Volume 4, February 2001.

Amara et al., Nucl. Acids Res. 25:3465-3470, 1997.

Arfin et al., J. Biol. Chem. 275:29672-29684.

Ausubel et al., In: Current Protocols in Molecular Biology, John, Wiley & Sons, Inc, New York, 1994.

Beaucage, Methods Mol. Biol. 20:33-61, 1993.

Chuang et al., J. Bacteriol. 175:2026-2036, 1993.

Coombes et al., Infect. Immun. 69:1420-1427, 2001.

15 Cornelis et al., Curr. Opin. Microbiol. 4:13-15, 2001.

Cummings et al., Emerg. Inf. Dis. 6:513-524, 2000.

DeRisi et al., Nature Genetics 14:457-460, 1996.

Detweller et al., Proc. Natl. Acad. Sci. USA 98:5850-5855, 2001.

Egholm et al., Nature 365(6446):566-568, 1993.

20 Feng et al., Proc. Natl. Acad. Sci. USA 97:6415-6420, 2000.

Fox, J.L. et al., ASM News 67:247-252, 2001.

Froehler et al., Nucleic Acids Res., 14(13):5399-5407, 1986.

Gillam et al., J. Biol. Chem. 253(8):2532-9, 1978.

Gillam et al., Gene 8(1):99-106, 1979.

25 Gingeras et al., ASM News 66:463-469, 2000.

Graham et al., Curr. Opin. Microbiol. 4:65-70, 2001.

Graham et al., Proc. Natl. Acad. Sci. USA 96;11554-11559, 1999.

Ichikawa et al., Proc. Natl. Acad. Sci. USA 97:9659-9664, 2000.

Itakura et al., J. Am. Chem. Soc. 97(25):7327-32, 1975.

30 Kagnoff et al., Curr. Opin. Microbiol. 4:246-250, 2001.

Khorana, Science 203(4381):614-25, 1979.

Klug et al., Methods Enzymol. 152:316-325, 1987.

Koshkin et al., Tetrahedron 54:3607-3630, 1998.

Koshkin et al., J. Am. Chem. Soc. 120:13252-13253, 1998.

Kricka, Nonisotopic DNA Probe Techniques, Academic Press, San Diego, California, 1992.

Liang et al., Methods Enzymol. 254:304-321, 1995.

5 Lockhart et al., Nature Biotech. 14:1675, 1996.

Maskos et al., Nuc. Acids. Res. 20:1679-1684, 1992.

Neidhardt et al., in Escherichia coli and Salmonella (Neidhardt, FC, Ed.), Vol. 1, pp.13-16, ASM Press, Washington, DC, 1996.

Newton et al., J Comput. Biol. 8:37-52, 2001.

10 Pietu et al., Genome Res. 6:492, 1996.

Plum, et al., Infect. Immun. 62:476-483, 1994.

Rappuoli, R. Proc. Natl. Acad. Sci. USA 97:13467-13469, 2000.

Robinson et al., Gene 148:137-141, 1994.

Rosenberger et al., J. Immunol. 164:5894-5904, 2000.

15 Sambrook et. al., In: Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Sambrook et al., In: Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 2001.

Schena et al., Science 270:467-470, 1995a.

20 Schena et al., Proc. Natl. Acad. Sci. USA 93:10539-11286, 1995b.

Shalon et al., Genome Res. 6:639-645, 1996.

Su et al., Molec. Biotechnol. 10:83-85, 1998.

Velculescu et al., Science 270:484-487, 1995.

Wahlestedt et al., PNAS 97:5633-5638, 2000.

25 Wei et al., J. Bacteriol. 183:545-556, 2001.

Wendisch, et al., Anal. Biochem. 290:205-213, 2001.

Wood et al., Proc. Natl. Acad. Sci. USA. 82:1585-1588, 1985.

Yoshida et al., Nucl. Acids Res. 29:683-692, 2001.

Zhao et al., Gene 156:207, 1995.

10

CLAIMS

- 1. A method for depleting or isolating a targeted nucleic acid from a sample comprising:
 - a) incubating the sample with a first bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the first targeting region and the targeted nucleic acid;
 - b) incubating the first bridging oligonucleotide with a capture oligonucleotide comprising a nonreacting structure and a capture region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the bridging region and the capture region; and
 - c) isolating the targeted nucleic acid from the remainder of the sample.
- 15 2. The method of claim 1 wherein the targeted nucleic acid is rRNA.
 - 3. The method of claim 2, wherein the rRNA is prokaryotic 16S, prokaryotic 23S, eukaryotic 17S or 18S, or eukaryotic 28S rRNA.
- The method of claim 3, wherein the rRNA comprises the sequence of SEQ ID NO:23, 20 4. SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEO ID NO:36, SEO ID NO:37, SEO ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEO ID NO:41, SEO ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ 25 ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEO ID NO:58, SEO ID NO:59, SEO ID NO:60, SEO ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEO ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, 30 SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.

WO 03/054162 PCT/US02/41014 91

- 5. The method of claim 1, wherein the sample comprises eukaryotic nucleic acid.
- 6. The method of claim 1, wherein the sample comprises prokaryotic nucleic acid.
- 5 7. The method of claim 6, wherein the prokaryotic nucleic acid is from a gram positive bacterium.
 - 8. The method of claim 6, wherein the prokaryotic nucleic acid is from a gram negative bacterium.

9. The method of claim 1, wherein the bridging region, targeting region, or capture region comprises at least 10 nucleic acid residues.

10

25

- 10. The method of claim 9, wherein the bridging region, targeting region, or capture region comprises at least 15 nucleic acid residues.
 - 11. The method of claim 10, wherein the bridging region, targeting region, or capture region comprises at least 20 nucleic acid residues.
- 20 12. The method of claim 1, wherein the bridging region or the capture region is polypurine or polypyrimidine.
 - 13. The method of claim 12, wherein the bridging region is polypurine and the capture region is polypyrimidine.
 - 14. The method of claim 1, further comprising incubating the sample with a second bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the second bridging oligonucleotide and the targeted nucleic acid.

92

15. The method of claim 14, wherein the targeting region of the first bridging oligonucleotide is complementary to the sequence of a targeted nucleic acid and the targeting region of the second bridging oligonucleotide is complementary to a different sequence of a targeted nucleic acid.

5

15

25

- 16. The method of claim 15, wherein the targeting region of the first bridging oligonucleotide and the targeting region of the second bridging oligonucleotide are complementary to the same targeted nucleic acid.
- 10 17. The method of claim 15, wherein the targeting region of the first bridging oligonucleotide and the targeting region of the second bridging oligonucleotide are complementary to different targeted nucleic acids.
 - 18. The method of claim 17, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of the largest rRNA molecule and the targeting region of the second bridging oligonucleotide is complementary to a sequence of the second largest rRNA molecule in the sample.
- 19. The method of claim 14, wherein the targeting region of the first or second bridging
 20 oligonucleotide hybridizes to a sequence located between 100 and 5000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 20. The method of claim 19, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 150 and 4000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 21. The method of claim 20, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 200 and 3000 residues of the 5' or 3' end of the targeted nucleic acid.

93

- 22. The method of claim 21, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 250 and 2000 residues of the 5' or 3' end of the targeted nucleic acid.
- 5 23. The method of claim 22, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 300 and 1500 residues of the 5' or 3' end of the targeted nucleic acid.
- 24. The method of claim 23, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 350 and 1000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 25. The method of claim 24, wherein targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 400 and 900 residues of the 5' or 3' end of the targeted nucleic acid.
 - 26. The method of claim 25, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 450 and 800 residues of the 5' or 3' end of the targeted nucleic acid.

20

30

- 27. The method of claim 26, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 500 and 700 residues of the 5' or 3' end of the targeted nucleic acid.
- 25 28. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence at the 3' or 5' end of the targeted nucleic acid.
 - 29. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 100 residues from the 3'or 5' end of the targeted nucleic acid.

94

- 30. The method of claim 14, wherein targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 200 residues from the 3'or 5' end of the targeted nucleic acid.
- 5 31. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 400 residues from the 3'or 5' ends of the targeted nucleic acid.
- 32. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.
 - 33. The method of claim 1, wherein the bridging oligonucleotide comprises a second targeting region comprising at least 5 nucleic acid residues complementary to a different sequence than the sequence to which the first targeting region is complementary.

20

- 34. The method of claim 33, wherein the first targeting region is complementary to a different targeting nucleic acid than the second targeting region is.
- 25 35. The method of claim 1, wherein the first bridging oligonucleotide comprises two bridging regions.
 - 36. The method of claim 1, wherein the bridging oligonucleotide or the capture oligonucleotide is RNA, DNA, LNA, iso-bases, or a peptide nucleic acid.
 - 37. The method of claim 1, further comprising washing the capture oligonucleotide after incubation with the sample and the bridging oligonucleotide.

- 38. The method of claim 1, wherein a) and b) are performed at the same temperature.
- 39. The method of claim 1, wherein a) and b) are performed at a different temperature.

- 40. The method of claim 38, wherein a) and b) are performed at the same time.
- 41. The method of claim 1, wherein the nonreacting structure comprises a bead comprising plastic, glass, teflon, silica, a magnet, cellulose, latex, polystyrene, nylon, cellulose,
- 10 nitrocellulose, polymethacrylate, polyvinylchloride, or styrene-divinylbenzene
 - 42. The method of claim 41, wherein isolating the targeted nucleic acid away from the sample comprises exposing the sample with the capture oligonucleotide to a magnetic field.
- 15 43. The method of claim 1, wherein the nonreacting structure is cellulose.
 - 44. The method of claim 1, wherein the nonreacting structure is biotin.
- 45. The method of claim 44, wherein isolating the targeted nucleic acid comprises incubating 20 the sample with streptavidin or avidin.
 - 46. The method of claim 1, wherein the sample, capture oligonucleotide, and bridging oligonucleotide are incubated in a buffer comprising TMAC or TEAC.
- 25 47. The method of claim 1, further comprising:
 - d) discarding the portion of the sample that hybridizes to the capture oligonucleotide.
 - 48. The method of claim 2, further comprising:
 - d) discarding the targeted rRNA nucleic acid; and
 - e) producing cDNA using mRNA in the remainder of the sample.

WO 03/054162

PCT/US02/41014

- 49. The method of claim 2, further comprising:
 - d) amplifying nucleic acids in the remainder of the sample, wherein the remainder of the sample is enriched for mRNA.

96

- 5 50. The method of claim 49., further comprising:
 - e) using the amplified nucleic acids to probe a nucleic acid array.
 - 51. The method of claim 48, further comprising:
 - f) attaching the cDNA to a solid support, wherein a nucleic acid array is created.

10

- 52. The method of claim 51, wherein the solid support is plastic, glass, or nylon.
- 53. The method of claim 52, wherein the solid support is a plate.
- 15 54. The method of claim 53, wherein the plate is a multiple-well plate.
 - 55. The method of claim 48, further comprising:
 - f) contacting a nucleic acid array with the cDNA.
- 20 56. The method of claim 1, further comprising incubating the sample with a second bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the second bridging oligonucleotide and a second targeted nucleic acid.

25

30

57. The method of claim 56, further comprising incubating the sample with a third bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the third bridging oligonucleotide and a third targeted nucleic acid.

15

- 58. The method of claim 57, further comprising incubating the sample with a fourth bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the fourth bridging oligonucleotide and a fourth targeted nucleic acid.
- 59. The method of claim 56, wherein prokaryotic and eukaryotic rRNAs are targeted nucleic acids.
- 10 60. A method for depleting rRNA from a sample comprising:
 - a) incubating the sample with at least a first (1) bridging oligonucleotide comprising a bridging region comprising a poly-purine region of at least 5 residues and a targeting region comprising at least 5 contiguous nucleic acid residues complementary to a sequence of an rRNA molecule and a (2) capture oligonucleotide comprising a magnetic bead and a capture region comprising a poly-pyrimidine region of at least 5 residues, under conditions to allow hybridization between the bridging oligonucleotide and the capture oligonucleotide and the bridging oligonucleotide and the rRNA;
 - b) incubating the sample with a magnetic bead; and
- 20 c) isolating the magnetic bead.
 - 61. A kit, in a suitable container means, comprising:
 - a) a capture oligonucleotide comprising a capture region and a magnetic bead; and
 - b) at least a first bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
- 62. The kit of claim 61, wherein the first bridging oligonucleotide comprises a second targeting region.

63. The kit of claim 62, wherein the first and second targeting regions have the same nucleic acid sequence.

PCT/US02/41014

- 64. The kit of claim 62, wherein the first and second targeting regions have different nucleic said sequences.
 - 65. The kit of claim 64, wherein the first targeting region is complementary to a sequence of an eukaryotic rRNA and the second targeting region is complementary to a sequence of a prokaryotic rRNA.

66. The kit of claim 64, wherein the first targeting region is complementary to a sequence of an eukaryotic rRNA and the second targeting region is complementary to a sequence of a different eukaryotic rRNA than the first targeting region.

10

- 15 67. The kit of claim 64, wherein the first targeting region is complementary to a sequence of a prokaryotic rRNA and the second targeting region is complementary to a sequence of a different prokaryotic rRNA than the first targeting region.
- 68. The kit of claim 61, further comprising a second bridging oligonucleotide comprising (1)
 20 at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
- 69. The kit of claim 68, wherein the targeting region of the second bridging oligonucleotide 25 is complementary to a sequence of the same rRNA as the first targeting region.
 - 70. The kit of claim 68, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of the largest rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of the second largest rRNA in the sample.

- 71. The kit of claim 68, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic rRNA and the targeting region of the bridging oligonucleotide is complementary to a sequence of a prokaryotic rRNA.
- The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic 28S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a eukaryotic 17S or 18S rRNA.
- 73. The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 16S rRNA.
 - 74. The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic 28S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA.

- 75. The kit of claim 68, further comprising a third bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
- 76. The kit of claim 75, wherein the targeting region of the third bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA.
- The kit of claim 75, wherein the targeting region of the third bridging oligonucleotide is complementary to a sequence of a eukaryotic 18S rRNA.
- 78. The kit of claim 75, further comprising a fourth bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.

100

- 79. The kit of claim 78, wherein (i) the targeting region of the first bridging oligonucleotide is complementary to a sequence of a prokaryotic 16S rRNA, (ii) the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA, (iii) the targeting region of the third bridging oligonucleotide is complementary to a sequence of a eukaryotic 18S rRNA, and (iv) the targeting region of the fourth bridging oligonucleotide is complementary to a sequence of a eukaryotic 28S rRNA,
- 80. The kit of claim 61, wherein the first targeting region of the bridging oligonucleotide comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.
 - 81. The kit of claim 61, further comprising a buffer comprising TMAC or TEAC.
 - 82. The kit of claim 61, further comprising a magnetic stand.

20

- 83. The kit of claim 61, further comprising:
 - c) a solid support for preparing a nucleic acid array.
- 84. A bridging oligonucleotide comprising a (1) bridging region comprising a
 polypyrimidine or polypurine stretch and a (2) targeting region comprising at least 10 contiguous nucleic acid residues complementary to a sequence of an rRNA.
 - 85. The oligonucleotide of claim 84, wherein the rRNA is eukaryotic.
- 30 86. The oligonucleotide of claim 85, wherein the rRNA is the 28S rRNA.
 - 87. The oligonucleotide of claim 84, wherein the rRNA is prokaryotic.

- 88. The oligonucleotide of claim 87, wherein the rRNA is the 23S rRNA.
- 89. A method for depleting or isolating a targeted rRNA from a sample comprising:
- 5 a) obtaining the kit of claim 61;
 - b) incubating the sample with the bridging oligonucleotide under conditions allowing hybridization between the targeting region and the targeted rRNA;
 - incubating the bridging oligonucleotide with the capture oligonucleotide under conditions allowing hybridization between the bridging region and the capture region; and
 - d) isolating the targeted rRNA from the remainder of the sample by incubating the sample with a magnetic field.
 - 90. The method of claim 89, further comprising:
 - e) obtaining the remainder of the sample enriched for mRNA;
 - f) preparing cDNA from the mRNA.
 - 91. The method of claim 90, further comprising:
 - g) constructing a nucleic acid array with the cDNA.

10

- 92. The method of claim 89, wherein the mRNA or prepared cDNA is amplified.
- 93. The method of claim 92, wherein the cDNA is used to probe a nucleic acid array.

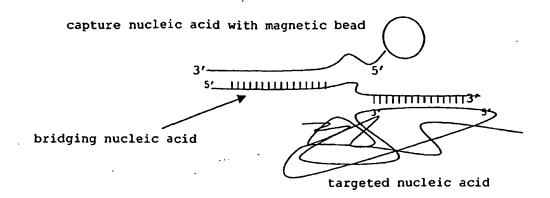


FIG. 1

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Welghted residue weight table. Tuesday, November 27, 2001 4:14 PM

One		10	20 30 40	or.	07-	. g.	- 09	
779.907 TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	, and a second s		U	94	Ė	٤]	
penturecisis. SEC								٥
Efacelia 165.520	CONVCCTV-COCC	E				· · · · · · · · · · · · · · · · · · ·		Ģ
Llactis 165.6EQ	-777474		CATG M		actat. Cac	a.gg.	JC.	'n
Lmonocyt166.8EQ	GCC70CAGACGACAAC			: ::::::::::::::::::::::::::::::::::::	8		9	9
Saureus 168.530	77. 21.00 CT		9	: ::::::::::::::::::::::::::::::::::::				'n
Smutans168.520			G		5		9	~
Spneumon168.8EO			g	2	8		\$	5
Spyogenes 168.520				: :: ::: :::				~
Mavium168.820					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	******	0	
Mtb168.820			****		g	E- 1		s
Ecoli o157 168 gan			g		6		9	-3
Can by the trouties of the		TCAACACTTTCATC	ATGCTCACA	TTCMOCCT	30000000	-cervacae	ATCCAROT 6	
Add tholes, suc		4	z				87	יש ו
Hinfluenzae 165.8EO	09				!		9	0
ronchiseptics 165.8			: : : : : : : : : : : : : : : : : : : :	:::::::::::::::::::::::::::::::::::::::		E .	9	ce
repertuesis168.820	Bparapertuesia168 SZO			:::::::::::::::::::::::::::::::::::::::	G.T.	7.7.	9	_
Spertuseis 165.520	Spertuseis 166.550	******		: : : : : : : : : : : : : : : : : : : :	0.7.	T.T.	ñ	10
Bospacia 168.520	61			•	Q.T	T.T.	9	
Brallet 168.820	63	•••••••••		•		- 4	9	-
seudomallei 16S.SEQ	75	***************************************		: : : : : :			£	_
Ngonorrhoese 168.520	Ngonorrhoes 168, 520 T			:::::::::::::::::::::::::::::::::::::::				16
Mentag 165.880	January 19. 19. 19. 19. 19. 19. 19. 19. 19. 19.			:::::::::::::::::::::::::::::::::::::::		7.4	9	
Paeruginosa 168.520	**************************************			:::::::::::::::::::::::::::::::::::::::		T.T.	9	~
Vcholerae 168.8EQ					¥	Crac	9	9
nterocolitica 165.8	Yenterocolitics 165, 520-N.						9	0

FIG. 2A-1

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table, Tuesday, November 27, 2001 4:14 PM

G=-GAT0G			110	120	130	140	150	160	- 5	
	C.CATOT	0.1		88	J.	PA. CT	Ü	9		166
. A. TT,0.A AG				8 8		222. G.	Ü	JC		164
70C.0.No.175	.TAC CACT	<u>ສ</u>	4	8	.C.CSGTTTCA	110.0.0				155
	cr.ccwor		•		oo.	TW. TTG.	Ü	0.0		184
**************************************	ACTOC ATOT	······································		8	COCO AT C A ATAA CT	ATA. CT.	F	9 C		175
AC.Q.AQ.AQQAQQTTCTC.Q.ATTA	CTC. G. AT-				.c.œ.aт.,c,Ar.A.c.g	AT. A. C. G.	fo f	4		175
Company of the second of the s			200 m		TCA	CA. C. Q.	[-	₹		
	T.OGGRGAT-		4	88	CHOSC.T.A.CTOCHCTTCGCTGT.GOT	TOCACTAC.	600	D. F	8	131
CANCOCINGCINGA——ACCITICITITIC——TGACAAGGGGAAGGGGGAAGAGAAAAGAAAAAAAAAA	CTICITIOC	TCACCACTOCC	GCACCCCTCAC	TAATOTCTOO		TOTAL TAC	F1000	7.0		0,1
66CAG	8.6					Ö			A. T.A. T.A.	9 4 7 2
	a			: ::		.TG.		J		173
Bbrenchiseptica 168.8EQ.GC.GC.G		9		: , 5: *	o `		0	JT.	:	175
Sparapertussis168.630 GC.GC.OG		b		; ·	3 8	0 0 K 0 K	•	8		20
	.000C.,NG,.G.,		4	<u>ب</u>		36.4.0.6		3 6		7
	, AC, G, G, A, ACA.C, CAT.CTG.A.T.G, G.CCGGC, GCCGGAR			X5X.C.	5	TO.A.T.G.	80.0	88	ล์) [
	German	E-		XX	ð	TG.A.T.G.	•	d.ccccccccur	2kT	14
. a c.a C.laa			4	T:.ACA.CGCA:.T.CTG.A.T.GG.ccccacccg.AT.	38	10.A.T.G.	6.00	203	. A T	233
dG.GCha,	8	0	Ą		8	30.3.1.0.	5 5	64.0. GATC		2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	.0.GrafG.AratG.AratG.A		•••••••••••••••••••••••••••••••••••••••			.0.A.T.G.	5	0		E
Yenterocolitatem 168,880,.0C.G.G.,NT.A.ACNG.GC.,N.					C	Q.AQ.	U.3.	A.		£.

FIG. 2A-2

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	-											
-	180 19	190	200	210	220	230	340	250	- 2	270	280	
Beubtilis168.880	G COTTOTTE	TTO. MC. OC. T. (OFFICAMENTAL	N. TOC	AC.GC.T.GTTCAAACATAAAATGGCTA.CCA.TTA.AGAGGGC.C.	3	0.000	E	-			
Banthracisi6.520	dA. TITIG.	TTO.AC.0C.T.(OTTC. M. TIEM	A000	AC.GC.7. GTTC-AA.TIGAAA GGCT GGCTGTCA.TTATGA GGG. G.C.	3	0.0.08	f	~	4	4 C C C	. ~
Etacalis 168.520			COLEM. OTHER	¥act	.C. CC. T. CONDA OTT OCTT COGNOTICTCATCACOGOT. CTA	3	3000 C	F	Y	~	C. G. A 27	. AC
LIACTIA 108.88Q	TT. WY		TTTEN. TTTCA	M.ATOCA	A. AC TITIDA. TITGAA. ATOCAA. TGCATCA. TCAAA AT	AT.	.000.1.1.	-	Α	Α	T. A. A. 29	. ~
	A.: 0. TAX. TO		CCAC. CITTITICAL	A.Atoor	.6. C. T. CAC. CITITICADA. ATGOTT. GOCTATC. TTA. A		.coagr.c	+	A T		.A G.A 304	•
Smitana168.830			oricon.ord	A. A. OCT	AC. G. T. GTICALA, GTGALA, GGT., TG-CTGTCA, TTATA, GAT., GGGCT, CT, AAT, A, T, A	g	.000cm.c		X	TA	.AA.0 294	•
Spneumon168.810	0.00	0.7.730C.7.	CATTE CITIES	A. Aricca	ALINCIE I ALANTE TITABAN AUGCANG GCARCA, T.GTA	3 6	1000,1.1.	F (33	hTA	29	_
Spyogenes168.820	SOUTH TO SOUTH	ACTA. OC. T.	TA TAKTTAN	200	A. CC. T. T.A. TAATTAAAA. GOODA. TG.—CT. CA. CA. CA. CA. CA. CA. CA. CA. CA. CA	\$ 6		H &	: '			ο,
Mavium168.820		T 3C. T.	-remendance	3	OC. FTCTICTOGIOGAM,TT (Y)C. OTG. G. G	e		•	ξ ς : : ς	.	A.A. 18	m.,
Mtb168.820	00CC.A.00	A.00. TOC.T.	-TCTT. TOOTOG	\$5.0cm	1.10c.11ctr.10c10dAAA.0ctr.20C.073.6	4		£				
Scolf o157 168.520	COCATALOGICOCAL	acmencenno	-AGGGGGACCTT		GACCAANG-AGGGGACCTTCGGGCCTCTTGCCATCGGATTGCCAATDGCGATTAGCGATTAACTGATGGGGAAACTTAAGTGAAAAAAAA	CATOTOCO	AGATOGGATTA	CCTAGTAGG	CCCCTAAC			
Kpneumoniae 168.820		,									mandature 401	
Aactino168.520	.0		-1.CT.	¥4			.70.04	į.	4	t		
Hintluenzae 168,520	OTTAO		-T.CTGM	B	. TO T. C TGAGA G. A A AG		. AG		*	٠. ئ	70. 7. 286	
baroncalespeace 108.820cocc.Tho			•			8	5.7C	•		₹5		
Sparsperedeatelesses and Cocc. TAC				1	T	8	3. T C	4		۲۲	6 25	
Bearacta 168.880			· ·		.000	8	J.T C		:::::::::::::::::::::::::::::::::::::::	, t	6 27	_
Brallet 168.530	10					2 1	0. TOOCT.	-	¥			~
Bpseudomallet 168.5EQCGATG.G	8		Ü		10C	2 2	3.100cT	H 1	¥	را ا		M
Ngonorrhoese 168.820	o.crata.		đ		MODE			H (۲			، حث
Nmening 168.580	OORCITO.	TO COLONO.	ฮ		1000Ch	3 8			4			·-·
Paeruginose 168.520	0010.110.0		-tc	A.	.000TCTA. ===A-====A	*	0.0	E	*		BZ 5%	~ 1
Vcholerse 168.8EQ	C.		5		0	4				٠.٠٤٠٠	82 D	M, .
Yenterocolitica 168.5EQTTCC		1100 · · ·	4	A	T				> F		No c	٠.,
									40000000		WY	

FIG. 2A-3

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	290 300 110 120 320 310 310	300	. 5	[•	<u>ا</u> ۽		 	'					-	-	
Den by to tely admin				;]		; - -	*	•	2	٥	. 220	380	390	00 ~	
799:00:01	C			9			:		A	0	2.0	À	0 0	2 4	404
Ben Chrecielo. 520	C.AC.	9	37.0	3					•						;
Efactalis 168.5EQ	C.AC.	9	e E			•				· ·	;			¥.	404
Liactis 168.820	טי אט		Ç			; :	:	:	•			· · · · · · · · · · ·	88	.A.C.	ä
Lappocyt168.820	2				: : :		: Y:	:::::::::::::::::::::::::::::::::::::::	¥		88		88	A.C	415
Saurenel 68 880		:		,		:	:::::::::::::::::::::::::::::::::::::::	:::::::::::::::::::::::::::::::::::::::	K		ç.o	AA.	r	.A.C	424
Smitanal 68 gm		, ·	9			::::	:::::::::::::::::::::::::::::::::::::::	:	Y		5.0.	A		A.C	414
Superince 1 de con		9	0.4	,			:::::::::::::::::::::::::::::::::::::::	:::::::::::::::::::::::::::::::::::::::	K			A. A		A.C.	413
Sworteness of any						ž	:	:	* ······	C	8	A G ?	r000	T.G	400
Management of the Company of the Com			D + (,		υ	: : : :	:::::::::::::::::::::::::::::::::::::::	¥	· · · · · · · · · · · · · · · · · · ·	383	.0	88		
Page 100 and 1 and 1	;			,		C	z: :::		•	•				F. G	
MED168.8EQ	0.0	9	J. 7 G			T.								5	
Ecoli o157 168.SEQ	TACTOSTCT	acacia	TENCOLOCCI	CACTOC	ACTORCA	Choosed		CTACCOCL	SOCROCIONO	COCA STATE				THOCTOSTICTURARIANTER CONCOUNTRACTIONAL TRACTIC CONTROL OF THE STATE O	
Kpneumomiae 168.630													7. T.	LAIGCCCCCTC	
Actino168.830			Ö	Ü		:	:	:	•	:::::::::::::::::::::::::::::::::::::::	:::		•••••	3.5	397
Minfluensae 168.822						:		:		:					404
Bbronchiseptice 168 520	5				:	•	:			•		0.0.0.		0.00 G.	406
Boarabertussisi68.800	£			•	:	;	:::::::::::::::::::::::::::::::::::::::	:					C	101	401
Boertussis 165.520				•	:		:	:	• • • • • • • • • • • • • • • • • • • •	+		. O C.			373
Boopacia 168.820						; t			• • • • • • • • • • • • • • • • • • • •			0		······································	399
Bmallet 168, SEO					:	; c	:	:::::::::::::::::::::::::::::::::::::::	•					207 · · · · · · · · · · · · · · · · · · ·	403
Boseudomalle, 168,820						ن :	:	:::::::::::::::::::::::::::::::::::::::				••••••			378
Mgonorrhoese 168 820	Ö		ر د	, (; (:	:	••••••					35°	454
Nmening 168.830	9					; c	:	:	• • • • • • • • • • • • • • • • • • • •						401
Paeruginose 168.820	•		f			;	:							£ £ £	407
Vcholerae 168.8E0					:		:::::::::::::::::::::::::::::::::::::::	:::::::::::::::::::::::::::::::::::::::	•		<u>6</u> .			40.	463
Yantarocolitica 168.820	2					:	:	:			• • • • • • • • • • • • • • • • • • • •	••••••	•••••••		404
				•			:::::	:::::::::::::::::::::::::::::::::::::::			•••••	••••••			406

FIG. 2A-4

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table.

Tuesday, November 27, 2001 4:14 PM

	410		430	440	450	460	67.0	- 8	- 63	- 8	510	ŝ	
Benbtilisi66.852 Banthracisi6.852	75.7		5.5	TOTTA	0 6	f. C CT.: G. TOTTA A CAA. T. C-COTTCG GOCCG. TA. C GTAA. CAG. A. GC	.0000.TA.C	0	TAN.CAD	λ.α.	γ.	1:	524
Efacalis 165.520	1 4		to.	TOTALA.) ð			0 0		A.86.	γ	:	522
Liadela 168.530 . Lmonocye168.880			X CTa	TO. TA. A.	8	A.CACTQ.TG.TA.AACGTT.GTG.GGGAGC.CATCA.GGA.TAC.CAG.A.G.GAGC.CATCA.G.	AGC. CATCA.	9	A. TAC. CAG	A.O.G.	.		531
Saureus168.830	,		9 E)4	TOTTA.A.	0 (**************************************			T.TM.CM	.A.OC		:	541
Smutens168.820	AGTT.	TT.	t	TOTAR TO	3 (. TATCE	. y. 8			531
Spneumon168.520	AG	TTA.C	ដ	TOTAL A	8	.CT. G. TOTAA.A	2 4 5 . S	9 0	S.TTA.CEC	. A. O. O.	G. G.TTA.CAG.A.G.G.	:	531
Spyogenes168.8EQ	75.	TTA.C.	g	. TOTTA.AA	J	A.CCTG.IOTIA.A.AATGAT.GTGGGGG.=.AA.CCA.CA.GA.TAA.CAG.A.G.GA	- A. OC. C.	Ö	1.2X.C6	000	4		518 426
799.901m1799.		• • • • • • • • • • • • • • • • • • • •	8	C.ATC.					Sold.		~		, ;
Reply olsy 168 gro	GGG . T C		8	C.ATC.	!		C.CT.GG.	0	Sono.		٠		513
Koneumoniae 168.820	S. C.	CTTC CONTROL	SAMOTINCTE	TCACCGCCAK	3	and the control of th	TACCTTTOCTCA!	TOTOTTA	200-00th	MANAGEGGG	activicacona	2000	523
Aactino168,880						" Charles Can Can Garage Con Canal C	A.C.CATCO.		•		• • • • • • • • • • • • • • • • • • • •	:	313
Minfluenzae 165.520	~		•		:	The state of the s	.G.A.GC.X		L -ta		•	:	521
Bbronchiseptica 168 groot, T	BOOC. T	•		TO THE		TT. AIG.			L-12		AA-TA	:	523
Bperapertussis168.820 .cc. T.	8		:		! :		T.C.010CL.	0			A	:	518
Spertussis 168.530	8				! ! :		.T.C.OTOCA.	9		GTT	A	::::	490
Bospacia 168.8EQ	9.		U	4 0 50		TOTAL C. D. D. C. C. D. D. C.	T.C.OIGCA.	0	E	-		516	916
Emalle1 165.580	9		U	4 0 55		TOTAL OF THE PARTY	2000 - 1-000 2000 - 1-000 2000 - 1-000			Ŧ		:	519
Bpseudomallei 168.5EQ .G	0					TOOL OF A CALENDARY OF THE COMMENSAGES.		0	0.A		dg.AT		435
Nganorrhoese 168.530						TOTAL TO THE OF OWN AND AND AND AND AND AND AND AND AND AN							\$71
Mmentng 168.820	0		C	4 60		G. TOTAL B. S. C. S. S. S. C. S.	3.00.00		Y . Y .			:	924
Paeruginosa 168.820	6	4	U		: 1 :		r. AGC. GCTG		. T.A.		A	:::	224
Vcholeras 168.830				F	:	The state of the s	5	:::::::::::::::::::::::::::::::::::::::	٠ ا		6	:	519
Yenterocolitics 165.820G.	300		,	•		The state of the s	AATCAT					:	521
			:				691100.						523

FIG. 2A-

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	-	ŀ	-	ŀ	-								
•	530	540	550	260	570	580	8 08.0	909	610	- 629	630	640	
Baubtilia16s.SEQ		4			5	5	νπς	-		 			;
Banthracie16.620		2	.15	:		Ö		£	y o	, , , , , , , , , , , , , , , , , , , ,	300	4 b	2 2
Lincels 168 SPO	:	ġ i		ei i		92	.TT	E	G			2	631
Latency 168.8EQ		1		F F	6 E				A 00. MOT.		ATT. T. TG	4	649
Saureus165. SEQ		8			E	9 0	2000:	F 6	00	H	.000	۲.	660
Smutane168.SEQ	:		.TCC.0OTC.	6	6	g	T. T. T. C. M. C.				51 3001TG	Z	650
Spacumon168.SEQ	:	.TTCC.0	.0oxcrr	H	:	2	. AG	£	7.00	£	AIA 7 CO A 645	ביים ייש	3 3
Ware the first SEQ		.T TCC.0.	•	•		X	orcrr.	.T. A	.TAT 63. ATT.		W.T. T. G-	< 4	\$ 4 A
Mebles are	E .	:		:::::::::::::::::::::::::::::::::::::::	:	AT.		C. TOTTC.	7.70	T 7	OT. AGCG.		59
Ecoli 0157 16S.SEO	GOOGCGGGGAAATACTS		. G GIC				**************************************	c. torne.	T.AC.		OT. MGCG C		632
Kpneumoniae 168.8EQ					10.35501.351	ARAKKINCALIK	THE THE PROPERTY OF THE PROPER	Venchante	MANATOCCOGO	GCTCMGC	TOCONCIOC	VICTGATA-	642
Asctino168.880		o	O								•	.TCA	634
Hinfluenzae 168.520	•••••••••••••••••••••••••••••••••••••••	Z	U	-			N			F (X		640
Bbronchiseptica 168.880	α,		• • • • • • • • • • • • • • • • • • • •			ą			•	H 6			
Bparapertussis16S.SEQ	+	:::		:		2	70	2		4 6			637
Bornacia 168.550			• • • • • • • • • • • • • • • • • • • •	:		р		X	Α	f			53
Bmallei 168.520						2 2	C.:A.C	λ .c.			:		
Bpseudomellei 168.520		-	b	o		2 2				:	•		
Ngonorrhoese 163.8EQ					:	8	03hACCAG.	ģ			•	40 - 10 E	690
Pastudinoss 166 680				:		8	AACCMG	:		***************************************	o	1.TCT-0.A	643
Veholerae 165.5mg				:	• • • • • • • • • • • • • • • • • • • •		dTCAGCCTT.	:				CA.A.0 635	639
Yenterocolitica 168.SEQ	Q						H				:		
								`			× .	-	~

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	- 059	. 099	670	- 89	, vo	069	700	710	72	,	730	740	75	750	760	
Baubtiliel68.520	8	8	A.A. 0		N			0	0		A.T. T. TOTA	-	T. TOTA			767
Banthracis16.620	a.a.	4.75	GCA.AA.M0.					A TIT. T. TOTA	U		4	£	TOTA		0	761
Efaccalis 168.520	9.0°		A.AG	-				A DI T T T	Ú		ŀ	į.	7.77		a	751
Llactis 168.820	7.6	85.0	. A.A O					A TOTAL	U			£	C. TOTA		0	269
Lanch continues of the continues of the contract of the contra	A.QAQ.	SCA.A.	GCA.AA.AG	•				0	U		A.T		T. TGTA.		Ö	780
Saureus168.82Q	AA	.0CA.A.					~	:	4		A. TTT. A. TOTA	í	100		£	770
Saucan: 165.520	T. T.	.CCA. A G A. A	3A.AG.					:	C. A.		A T.T. T. TOTC	£	T. TOTA		C	760
Spneumon168.SEQ	TIT. A		OCAMO . O . A.A O						Ü	:	A T. T	£	CTTOTA.		0	756
Spyogenes168.820	X0.X	•	OCA.AOA.AG	:					ט		A. T. T. T. TOTA.	f	T. TOTA		U	664
Mavium165.8BQ	.O	, MCT.C.	ACT. C. 1 G. N. N. 1 G. 1 G. 1 G. 1 G. 1 G. 1	F		6	Ö	T A	Ü		8	H	G.AGTA.	:	0	71.1
Mtb168.820	.aa.	MCT.C.	. ACT. C 9 A. AC. 0	+		9	Ü	J			8	H	G. AGTA.		4	752
Zcoll o157 168.8EQ	CTOCCHOCTTON	ACCOUNT	i¢ttottaalaasasatkalnittoelastsiksessytalnatsestalaitetaalaskathetasasasikessyasseseessesseestaskes	MADER	SOTOTAGO	COTCAMATA	COSTAC	CANCIDGA	GALTACO	SOTOCOLU	DOCOCC	200	NOCONCA	ğ	1000	762
Kpneumoniae 168.520	G								:	•			¥			754
Actino168.820	00010		ACTT. N. O.A N	•							٠.,٧		CONTOT.	0	ļ	760
Hinfluenzae 168.820	OT.AA	•	ACTT 0. A		g	:			. :	*	Α	ŀ	GANGT.	•	f	
Bhronchiseptics 168.880.AC:00A	D.AC.00A		AG	8	Z	•				Α	4		CAT. C.		C	757
Bparapertussis165.8BQAC.GGA	X3	.0.orc.	AG	8	:	A		TGCCATGATC.	Ü		A		QKT.C		£	-
Bpartuesis 168.550	XC.00		.G.OTCAG	8		A			Ü	.A	γ		GAT. C.		7.0	755
Bompacia 158.820	Q			3		λ		G		.A.	γ		O.C. T.	:	T.C	758
Bmallet 165.5EQ		O.C.		•	KC	A		9	Α	٠٠٠٠٧.	¥		a.cT.	:	D.F.	731
Bpseudomallei 165.520GA		A.O.C.	K	:::	:	:		A		. A.		•	0.0.7.		T.C	810
Mgenerrhoese 165.8EQ Gran. C	order. c.	.c.orc	AG	•	:	A		9		λ			GAT. C.	:	7	763
Nmening 168.520	OTCH. C.	.0.01C	•		::::	A		9		Α	Υ		.ATQ.CC.	:	J T. C	763
Pasruginosa 165.580	A	-		D. E					Ö	A	Α		-151	:	D	757
Vcholerae 168.520		Ę	••••••	Ę.	•			Α		:			7.0%		Ą	260
Yenterocolitice 168.826	50		•	:			:	24					4	: :		762

FIG. 2A-7

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	770	780	- 6-	- 80-	810	820	830	840	950	098	870	880	
	•	0				ASTO	2	.A.T.T.A.G.GGTC-CCCC.C.TAG.G.T.CT-	0000.C. 200.C	3.T.CT	A. N. CLCT.	ទ្រ	181
Banthracis16.520	• • • • • • • • • • • • • • • • • • • •	•	• • • • • • • • • • • • • • • • • • • •		• • • • • • • • • • • • • • • • • • • •	AOTO	7.7	. NOTO NA. T. T. A. A. OGT C-COCC. T. TNO. G. T. A T	COCC. T. TMG.(3.T.A. T.	ACACT.		980
8			•		•	Non	X.T.	. NOTG NA. T. T. G. A. GGT C-CGCC. T. CAG. G. T. C	COCC. 7. CAG. (3.T.CA.	λ		870
	_	*********	***************************************			MORE	A.AT.	AGTGA.AT.TAG.QAG.TA.AA.TTC.CTG.ATCAA.	-A.TTC.CTG.	NT. C.	***		887
Imonocyties.820 .	• • • • • • • • • • • • • • • • • • • •		•	:	• • • • • • • • • • • • • • • • • • • •	AOTG	Z.T.	AdrgAA.T.T.A.G.GGTC-CGCC.C.TAG.G.T.C	000C.C. TMG.(J. T. C	Α	L	899
Saureus168.8 <u>80</u>	• • • • • • • • • • • • • • • • • • • •					STOK	X	. AGTG AA. T. T. A. G. GGT C-COCC. C. TAG. G. T. C	COCC.C.TMG.(:	:		. 688
	-				•	AOTG	A.T.	AGTGAT.T.A.GCTC-CGGC.TAG.GAA	∞œтив.(:	:		888
			F9			AON	. A T.	.AOTGAT.T.A.ACTC-CG.,OT.TAG.GTA	CGOT. TMG.	F	:		875
2		••••••				AOTO	. A. T	AOTGAT.T.A.GCTC-CGGC. TAG.G	∞∞πω.				783
ន្ត	.00.	:	•		X	.007	. A . T.		T C	9.1	A		831
	g					.c0	A. T.		TT CA. 000.	J			872
•	GGGANGGGGGGAAGANTEAGANTEAGANGAGCTGGTAGTAGCGCGGGGGTAAACGATGTGAGGTTGGGCGTTGA-GGGGGGGTGCGGAAGCTAAGGGTTAAGTGAAC	acuncua	ATTROVENCY	TOSTACTOCT:	ACCOCCEMAN	CONTOROR	CTTOQUO	orresection	GOOTOOCT	TCCCCCCTANK	COUNTING		. 088
. 820		•	• • • • • • • • • • • • • • • • • • • •		•			**************************************		• • • • • • • • • • • • • • • • • • • •	Α		872
Asctino168.830			••••••			9	0	TGTGTGT	.c.c		T.A.		876
Hinfluensae 165.550		******	· · · E · · · · · · · · · · · · · · · ·		T				ACTC	E4	T. A. A.		. 878
Bbronchiseptica 168.552	3		••••••		c		t) ₹			20.02			874
Bperapertussisi68.820 A	A		•••••••		o	A	A.C.		HC. T.G.				846
Bpartussis 168.52Q	A				•	A	t v		C.T.G.	•	0	E	872
œ	λ		• • • • • • • • • • • • • • • • • • • •	••••••		A.	A. TT.		C-TT.C. ING.	:	υ		875
Bmallei 168.522	»	•	••••••	• • • • • • • • •		.A	A. 77		P-TT.C. TMG.	:	0	f	848
^	A		•				A. T.		T-TT.C. TMG	:	8	F	927
Monorrhoese 168.820	G					A.	T. A. CJ.		TIOC. T.O.	X63			881
Mmening 168.SEQ	G	********				A.	7. A. CT.		TTOC. T.O.		G A. T.	:	881
Patruginosa 168.520	•••••••			*******	• • • • • • • • • • • • • • • • • • • •	•	8		ATC, TAG.	80.0.08			875
Vcholerae 165.8EQ	•••••••••••		• • • • • • • • • • • • • • • • • • • •			£4	:					λ	878
Yenterocolitica 168.820							:::::::::::::::::::::::::::::::::::::::			•			880

9/54

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	- 68 -		- 00	910	920	93.0	040	950	096	970	086	086	7000
Beubtilisi68.520		f.	, KC 0.	0			4						1000
	••••••••	٠		9					4		E C		•
Efacalis 168.520 .	C	:		9	•	• • • • • • • • • • • • • • • • • • • •			4		4		E
	K	Α		9					*		4		A 7 10
	~·····	:	0	9	•	••••••		•	Α		Α		7 10
Saureus168.SEQ .	C	:		9	:				γ		***	4	7 100
	£	A		0							4		C 100
Spaeumen168.52Q	K	A	B	9							4		
S.	4	¥		9	:	••••••		•	Y		4		C06 D
Mavium168.8EQ	•••••••	N		0	:	N						ŧ	
Mcb168.650		-		9			9	4			Ē	ŧ	
	OCCITO COLO MATRICO		CUGGTTAM	ACTOUATE	WATTOACCO	OCCOCCIC	MCCOOTGCA	2CATOTOGET	OCOCINGATIANACTICANTICAL COCOCOCOCACA ACCOCACA ACCOCACA ACCOCATA ACCOCACA ACCACACATA ACCOCACA ACCACACA ACCACACA ACCACACA ACCACACA ACCACACA ACCACACA ACCACACA ACCACACACA ACCACACACACA ACCACACACACA ACCACACACA ACCACACACACA ACCACACACACA ACCACACACACA ACCACACACACACACACACACACACACACACACACACACA	ON RECOCCION C	PACCETAGO	CONCENCAC	ATTOCK 100
. 570	• • • • • • • • • • • • • • • • • • • •					•							
					:	•••••••	••••••	••••••				Ŋ	986 0
Hinfluenzee 168.820 .	•		• • • • • • • • • • • • • • • • • • • •			x	•	• • • • • • • • • • • • • • • • • • • •			.yc	:	866 2
Abronchiseptica 168.8EQ		E .	λ	0	••••••			70A		~		8	
Sparapertussis168.520			A			¥		.TG		Α			
Bpertussis 168.820		£-	γ				•	.AA.	•	~····	• • • • • • • • • • • • • • • • • • • •	:	
~	•••••••••			9	¥			A	••••••	~		88	oor 995
Emallet 168.820	•••••••••		A	.0	:	A		.TGA.	•	A	:	Acc	oor 96
Bpseudomallei 168.52Q	•••••	F	٠٠٠٠٧٠٠٠٠			AA	:	b.	• • • • • • • • • • • • • • • • • • • •		:	204	18
Ngonorrhoese 165.5EQ	••••••••••••			9	:	A						£	BR
			A	9	•	.					• • • • • • • • • • • • • • • • • • • •		£ :
g		:			•						• • • • • • • • •		d.T 995
Vcholege 168.820			A		• • • • • • • • • • • • • • • • • • • •	и	• • • • • • • • • • • • • • • • • • • •		Z	• • • • • • • • • • • • • • • • • • • •		Q.	95
Yenterocolitica 168.520			•			x			•		• • • • • • • • • • • • • • • • • • • •	Ş	3000

FIG. 2A-9

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

		201	1030	1040	1050	1060	1070	1080	1090	1100	1110	
Benchings 820 Banthraciais 830	-70. AA.C.TA.QAC-, CT702 G-, A.A.TGT	A. CBC-	T00	.0A.A.T.	0	e		00				111
Efactalis 168.820	7-70-CAC.77A., QCTT.C-, C. 703 d. 222.77.	A. 0071	C. C. 765	AGA-A-T	0	÷ •	•	g	:	•	9 111	0 1117
Llactis 168.520	- GTGCTNT-C-T. T-GC, TGGT-T	A. G.M. T.	8	, O O		F		: ::::::::::::::::::::::::::::::::::::		•••••••		. 1107
Lacnocyt165.520	T-TG. CMC. TG CM. GCTT. C 6. TGG. T G. AAA. T.	.COCTT.	CC.300	. AX. 0	c	F	:	· · · · · · · · · · · · · · · · · · ·	•	•	Ď	
Saureus 165. SEQ	T-TGNCT	A000T.	6.0.70	D. AM. T.	C	F	•	; ; ;			•	
Smutanai68.820	0.10CM.T.CTTA.QM.T.A.—C.10G.1MC.1C.—	A. C.A. T.	A-C. 786. 1	. YC 1C -	C	F	:		:	•		
Spneumon165 . 520	1010	.A. O. TT.		XC. 63 7.	c	F				•		
Spyogenes 168, SEQ	G.TCC.CCCT.	. A. O. TT.	A-C. 700. 7	JC 77 - 7	ď				• • • • • • • • • • • • • • • • • • • •			. 1112
Maviumi68.520	6C-coorcicar.cocrC.ror	.0AT.00C	T. C. TOT.	Cronono	c		:		: : : : : : : : : : : : : : : : : : : :	•••••••	Ö	. 1020
Mcd168.820	6c-conter:	CAT.OCC.	r. c. ror	W. 620. T. C. 100 Crontone				g	• • • • • • • • • • • • • • • • • • • •		•	9901 5
Ecoli o157 168,5EQ .	CAGNCT-TTCCACA	ATGGATTGGT		GRATICATION CONTRACTOR				a		•	¥:	G 1107
Kpneumoniae 16S.SEQ	THE THE PROPERTY OF THE PROPER				Y 1516	ALTOTOPIC ST	Accidate	TOTOLLAND	TOOOTTANCE	SCOSCANOGAG	CCCNCCCT	A 1117
Asctino168.880	# D	£		E			: : : : : : : : : : : : : : : : : : : :		• • • • • • • • • • • • • • • • • • • •	••••••		. 1109
Minfluenzae 168.820	•	Ş		Ē		X.						1113
ronchiseptica 168.	SEGO. TC-CCAR. Troo A.A. A. C. a. C.	TOOOL	100		:					•	••••••	. 1115
Bparapartussis165,520 70, 70-000h. Troon. Tree A. A. A. A. C.	0 TO TC-CO.	TOOOL	1 11 22				:::::::::::::::::::::::::::::::::::::::	Ca	• • • • • • • • • • • • • • • • • • • •	•		6 1113
ertussis 168.5EQ	70TC-000.	Troops	A AA COT	6				g	:::::::::::::::::::::::::::::::::::::::	•		G 1085
Bospacia 168.820	.atc-c.a.r.	G TOOOL	TOTAL A	TURANA A C. GATE			:::::::::::::::::::::::::::::::::::::::		••••••		•	0 1111
Brallei 168.820		T000C	TCTBBB.	TOTAL A COMP					: : : : : : : : : : : : : : : : : : : :	•		0 1114
Bpseudomallel 168.529 .0 CC-congr Gr	0 .0c-coat, grande Archan A C core	GTOOC	TOTALL A	PROGOC. TOTALAN A C COC					: : : : : : : : : : : : : : : : : : : :		0	0 1087
Ngonorrhoese 168.5EQ						• • • • • • • • • • • • • • • • • • • •			: : : : : : : : : : : : : : : : : : : :	•	116	g 1166
Numering 168.880				0				o	: : : : : : : : : : : : : : : : : : : :			0 1118
Pacruginosa 168.5EQ G	0						:::::::::::::::::::::::::::::::::::::::	g	: : : : : : : : : : : : : : : : : : : :	0	•	0 1118
Vcholerae 168.8EQ	GTCNG.GC.	_		CTGGA						Ē.	9	G 1112
Yenterocolitica 168.820Gr10	SEOG. T NO.	£	N . N	•			•					. 1115

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

•													
	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210	1220	1230	
Baubtilisi65.520	ATA.	Α	AT.A.TT.	cTTGCC	O					C.	Ę		1232
	AT	T.A	.T.W.TT	TM.T. CTTGCC.	9		•			C			1235
8	y	T.A	.TTA. TT	.ATTA.TTCT.OC	9							•••••	1225
	.TO. A T.	T.A-	.ATAA.TT	.C	9	0			.TT	C	£-	:	1242
	ATTA	A	TA: T	.CTA.TGCG.C.	9	C. G.C				C	4	•	1250
	30AT.)	T.A-	.TXT	.ATAA.TTCTTTGCC	9					C	TTT	: : : : : : : : : : : : : : : : : : : :	1246
	A	T.A	.TM.TTC	C7,8	.T.GC	AC			A.		E	•	1243
	.TGA		T.A. TT.	T.A.TTCT.CCGA		AC			A		£		1230
Spyogenes168.8EQ	.TGAT		.A, TAA. TTC T. GC.	CT.0C		GAC				.C7			1138
Mavium16S.SEQ			. 0 ME 0 OTCA	aora	9.5	6.6. TC CTC				C	F	A	1186
McD168.58Q	ฐ.	X	Mr.G.	.AcAAT.OT G OTCA	0.5	6.0.TCCTC			• • • • • • • • • • • • • • • • • • • •	.C		Α	1227
Ecoli o157 16S.SEQ	recritori	¥89508	TOCOCOCC	INCICAMOGN	HCTGCCAGT	GATAAACTGGA	DCANGOTOCK	XXXXXXXXXX	MATCATCAT	DOCCTTACC	co-atocsaccesalc texinosisictecisterisixinctesinosinosias cortexisterterioscettinoscenoscetizerenosteti	CACOTOCTA	1236
Kpneumoniae 168.520	• • • • • • • • • • • • • • • • • • • •		T.A										1228
Actino165.520			100gT.	MOOT. T			•		•	8	8	•	1232
Hinfluenzae 168.850			ACTT . T						• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	5	•	1234
Bbronchiseptica 168.520.AAT	Q.AA	.1.	XX							7.837		ช	1226
Bparaportussis165.880AAT				AM C T T		.GT.CC	• • • • • • • • • • • • • • • • • • • •			-	T.007.	ฮ	1198
Bpertussis 168.8EQ	. A A				9	GCC.	•	,		-		ថ	1224
Bcepacia 168.SBQ	AT	x	CAA.A.C.	.CT	9					T.00.T.	.T	ฮ	1227
Emallei 165.5EQ			- CAN.A	CM.A.CT		gc		• • • • • • • • • • • • • • • • • • • •		-	:	5	
Bpseudomallei 165.SEQAT.			4.89	C. C. A. C					C	T.00T.	.T	:	
Ngonorrhoese 168.820AA	AA	T.A-		.ATTTCTT.	9							5	1236
Nmening 168.520			T.A.TT.	.AT.A.TTCTTGCG.C	9						E	ฮ	1236
Paeruginosa 168.SEQ	AA	-		T	3	gc.			• • • • • • • • • • • • • • • • • • • •	.0		•	1231
Vcholerse 16S.SEQ	5	٠	Ac. AMT. CT.		0	0			••••••	5	5	•	1235
Yenterocolitica 168.820	Ωα		AC. ANT.OT.		9						0		1237

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1240 1250	1	1260	1270	1280		1290	1300	1310	1320	1330	1340	1350	
Baubtilis169.520	DCX		M C	Ė	5	C. A.C.	TOTOT.	g.	r c. c.		0.0	.CAACGTTCA.T.C.A-CTCTGTTCTC.C	0	1351
Binthracisio.SEQ Efaccalis 165.5EQ	מששט	ម្រ	A. C.C.	8 8	4.4	4 6		8 8	H		.CA.	.CTA.GRC	c.	1354
Llactis 163.550	ATOG C.		อ	TOTAL	1		ACC 4	į				TCGEC.H. T. TOTTT TA. T.		
Lannocyt168.520	D		700	8	Ę	Ą	ACTAT	ų				.TC NOC (GTGG., ZA, Z, C, A,, ACTAM_ TC T G		
Saureus165.SEQ	ACA		NC	b	XT	۲.5	5	ų	1		4	.c Mcor	•	2007
Smutane168.820			8.0.	8::8	TA.T	4	1000	Ł	T	0	Ú	(TT 0.00.T 00		1362
Spneumen163.8EQ			A.0.00.	8	T.AT.	-	8	Ę.	T T.	.0.	.CA.	TC. A. 0. CG. T CG TA. T T	Ü	1349
Spyogenes 168.880		a.	.A.0.00	8	T. 41	Ę	S	2	TT.		.CA.	TCA. G. OG. T CG TA. T T	C. 125	1257
Mavium168.820	D	g	130	₹.	T.A. T.	Ė		'n	ra		U	.cr10crhorr	đ	1306
Ntb168.520		o.g.	380	E	A.T.		8	ħ	0		Ö	.cr10c	5 6	77.
Ecoli 0157 165.8EQ	CATOCOCA	DCMADIONE	ECONOCIOS	SAGAGG	AGCOGAC	CTC-AT	WORK		COCATTOCA	GICTOCAACH	KACTECATEA.	CANTOCCAMPICANAGARACCICOCCAGARACCACTC-ATANAGACGTCGATTTCGATTTCGAATTCGAATTCGAATTCGAATTCAATTTCAATTAATT		1 2 6 6
Kpneumoniae 168.8EQ	AT	••••••		:			AT							, ,
Actino168.850	00	00.T.	1.C.A.G.	8.8	T.A.T.	9		4				.T.A.C.A.CA 72.00.7. A.T6.		, ,
Hinfluensee 168.880	TGG	00	ACC	999	T. K		4	4					; ·	707
Bbronchiseptica 168.8EQTCGGGG.	SEQTOOO	300.10.	C.A.C.	8	ð	Ü	8		i i		. TC C. A. C	• • • • • • • • • • • • • • • • • • • •	; :	
Sparapertussis165.530 TCGCCC.	DT000	300.70.	C.A.C.	8	ð	0	8		Ü			.166		3 .
Spertussis 168.SEQ		300.TT.	MC.A.C.	8.0	5	Ü	88	z	Ü			.TT.NC.A.C 0.00 0.4.T.C 0		
Bospacia 168.522	TOOCHOO	100.TT.	.TTC.A.C	9.0	T. AT.	00	ğ		Ü	U	C	.6.0013.1.C63AC.3	; ; ;	
Bmallei 165.5EQ	TCGGA0.0	100.TC.	C.A.C.	8.0	5	00	XXX.		Ü	Ú	Ö	.10C.A.CG.GGCA.T.CGACCGA.		
Bpseudomallet 165.580TCCCAGG	DOOL 0	100.TC.	C.A.C	00.0	5	00.	XXX		0		Ö	-10C. A. C		100
Ngonorrhoese 168.820	•	00	c.xoc		5	D	ACCC	•			.TC.NOCGCGGCA.TCNCGA		đ	
Maching 105.820	000.0.	0.0	c.yoc.		5		8	:::::::::::::::::::::::::::::::::::::::	G			• • • • • • • • • • • • • • • • • • • •	้ อ	1355
Feeruginosa 168.8EQ		£ 0		8	TA.T		8	:	C.C.	•••••••••••••••••••••••••••••••••••••••	0.0	.TTC. AGCTA. T. C ACCAA	• • • • • • • • • • • • • • • • • • • •	1350
Venotates 168.650	D		TMC	ğ		٠ <u>٠</u>	A	:		• • • • • • • • • • • • • • • • • • • •	••••••	.c TMC 0100 A. TC A.	5	1354
I du cer occitate de la seguina de la company de la compan	350 MG.	T		:::::::::::::::::::::::::::::::::::::::		: 	ະ			••••••	~		•	1356

FIG. 2A-12

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	ŀ								l	I	I					
	1360	1370	1380	1390	1400	1410	1420		1430	1440		1450	1460	1470	ا ه	
Baubtilie16S.SEO	0,			22 44 EL 4 C			4	1	Į	$\left \right $			1		;	
Banthracials, 820	·							. .		•				:		
Transle la con			•			, .		.	:	3	3		5	8C.1.30	•	
		•	:::::::		::::::	•	F	₹	:	3	8	C GAGG TITIG. AGCC	ဋ		1449	
	Y		:::::::::::::::::::::::::::::::::::::::				CTG.T.00C.	5	g	£				C.T.300.	3. 1478	
~				······6·····	:		C.ATTAACCC.	TX	•	Ü	8	.C		COC. G. AGG		
Saureualés. 520				A.F.	:		C. A TT A. A 00C.		:	S.	2	CC G. MG - TITTING BACTER COM G BACK	Ę	04 0 70		
		0	:::::::::::::::::::::::::::::::::::::::	:	:	•	C.AT	.TTAOCC.	:	.0.00	:	Trans seem cos	ACC)	OK T 200		
Spneumon168,820		:	••••••		:::::::::::::::::::::::::::::::::::::::	•	C.ATTA000	TX.		9	•	C GAGG G AAG. AGCCA CGC. T. AGG.	5	CC. T. NO.		
Spyogenes168.8EQ	CC.		•	•	:		C.ATTXACCC	X								
Mavium168.52Q	C.MCTG			• • • • • • • • • • • • • • • • • • • •		6		\$	×	g	Ü	.6TACACCAGCC. TTT.G.AGAGT.G.AGG.	, P	O. C.	• • •	
Mcd168.850	C.YCT			67. AA. CC. CCA. G. C. CCA. G. C.	:	5	3	\$	g	g	Ü	ט	9	0		
	ATCHAN-TOCU	TOCCACOGTONALA	COTTCCCC	COSTONARIOSTICOCOSOCCITOTACHOCOCCCOTICACHISOCALITICO ANNO ANTINA ANTINA CITARIO ANTINA CITARIO ANTINA CITARIO A	ACCCCCC	TCCCCC	COCHOTIC	Serrece	ARCAN.	TAGGER	CITABO	CTTCCCCAC	3333			
	.H			•••••••	:::::::::::::::::::::::::::::::::::::::			•								
	AT	bt.	•	T. C.				F	y	4	U	600		٤		
	A		:::::::::::::::::::::::::::::::::::::::	· · · · · · · · · · · · · · · · · · ·	:			8	Я	Y		8		C		
Bhronchiseptica 168.5EQCT.C	c.		:::::::::::::::::::::::::::::::::::::::	F	:			8	Х	e E	Ú	OCA . 0 co	8	C		
Bparapertussis166.850C T.G	-: O:		:::::::::::::::::::::::::::::::::::::::		•			3.8	:	F	Ü	T. C. OCA, 0.00	8	O		
Spertussis 168,820	C4.0		:::::::::::::::::::::::::::::::::::::::					4.8	:	TC		.OCM G. OG A	- B	8		
Boepacia 168.830		g	:::::::::::::::::::::::::::::::::::::::		:			48.8	Я	.0.CTC	3	.OCM0A 0.C.	0¥0	:	.00. 1463	
Bealle: 168.880		0	: : : : :		:::::::::::::::::::::::::::::::::::::::				:	. a.c TC.	:	.CCM0AG.C	0AG	:		
Bpseudomailei 165.5EQC.~G.									:	.o.cπ	:	.OCMGAG.C.	G7G	:		
Monorrhoese 168.880	.0C A. TO	.A. 73	•					CONT.CC.	:		:			:		
Mening 168.850		.gcλ.τσ						OGAT. CC	8		3	GAACAATC	 	0		
Paeruginosa 168.820	A	Å		•			•	F	8	Ü	Š,	. SCN G.	DA	0	CA 1467	
	A	λ	: : : : : : : : : : : : : : : : : : : :								+	1466	A	N. N.	1466	
Yanterocolitica 168.889						2		:							1473	

FIG. 2A-13

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

1540	T T T T T T T T T T T T T T T T T T T
1530	20
1520	16. CM2. T 16. CM2. T 16. CM3. T 17. CM3. T 17. CM3. T 18. CM3. T 19. CM4. T 19. CM4. T 19. CM3. T 19. CM
1510	N N N N N N N N N N N N N N N N N N N
1500	ATTOO TO A LOCATION CO.
1480 1490	. G. CAG T
i i	Beubtilisi69.5EQ Benthraciel6.5EQ C.CAG Eleacalis 168.5EQ Llactis 168.5EQ AACCG Lactis 168.5EQ G.CAG Saurens168.5EQ G.CAG Spheumon168.5EQ G.CAG Spheumon168.5EQ G.CAG Ratinol68.5EQ G.CAG Roll 0157 168.5EQ G.CAG Roll 0157 168.5EQ Actinol68.5EQ Actinol68.5E

Decoration 'Decoration \$1': Hide (as '.') residues that match Ecoli o157 169.5EQ exactly.

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

50 60 70 80 90 100 110	G. AGG. A.AG T A.C. AGGTG.T A	100	ACT: A. GA TT. TAG CA A. T GCA. TG. T CTA G.		TACT.A.GATTCGCA.T.ACCTG.TA.A						NTOCOCTOCLOTCHANGGO-ATANAGALCTGCTAATCTGCGATAAGCGTCGGTAAGCTGATATGAACCGTTATAACGGCGAT 112			fGACAd0CATd0GC0C.AAC.ACA.TG.TCA 113					•	Th. cc	A			4
	A.C.AGC. T	A. T. MGC T	100	C.T.308.T	A.T.AGCT	A.T.AGCC.1	A.T.AGCGAT	A.T.AGGGG	C. ACCOMOCA. 7	rc. Accessed o	KINDAMCOSTAN		A80	C.AMC.MCA.1	X.MC.MCA.	C. AMC. AGCA. 1	C. AMC. AGC	C. MCGNGC1	X.AACGAGC	Z.AT MGC. M	2C. AT ACCAN	x. Mc.akc	J. A. G.	
	83c.	3 5 5	S 8	f.T00C	F.T. 90. C.	f.T86C	2.T. GT. C	T.T00C	C00	C	STOCOTANOCTO	• • • • • • • • • • • • • • • • • • • •	T.G.A.GTC.	305	7508C.	70000T	TA00C	700c.	TA00C	88	.86.	T80TC.	ðv.5.	
- 2	A.ACT	A. 60	5		A.GAT	A.GAC.T	A. CA T C	A. GA A. T	•	X	VICTOCCATAAGO	A	A	X	χΑ	A	A	,			Α	XX	:::::::::::::::::::::::::::::::::::::::	4
		6 ACT		:	:			:	3.88	3.88	Machoritan			3		:)b.n			:		A	Α	
40 50	.T	T - ACT CA TO	-ACT. AGA 86	CTGAGG.A.	ACTACO	ACTGAOG	.TMCTChCG.C.	TACTA 03.A.	CO.C	CO. CO	TCAGAGGCG-ATC		A	c	.G		C	c	.c	.G.T	.G.TC			•
30 4		X- X- 1	A	TXCTGAOG.A	¥	AAI	A	₹-···₽···	TA., GAGA., CG	TACAGACO	итосостосско		TA	5	§		TGAC	4	₹	ðt	ðt	t	35	Ē
- 20	8.6	8	8.0	0.8	.0.8		.a.cd	8.5		T.T 0.00 T	ACOTACACISOTIC		T	T.C.T.T	.T.C.T.T	.T.C.7.7.			.xc1.c.101	.TA T.C.TCACTCA.G.TC	T. A T.C. TCA	C.T	• • • • • • • • • • • • • • • • • • • •	
- 01	5 6 E E	4	4.5	4.6	11.1	4.4.	T. A	TT. A	7.77		TEMOCONCENA					•	- 1	:	:			.CTMG	£-	ť
			5	20		• • • • • • • • • • • • • • • • • • • •			TOT. TOTANO	- TTOEA-	0 OTTAK		TATA	EQ A	EQ A	A.C.			0		180 OEI		••••••••	SEG
•	Baubtills 235.550	Efacaelle 235.5EQ	33.SEQ	Lamonocytogenes 23S.SEG	135.SEQ	13S.SEQ	Spneumoniae 238.SEQ	Spyogenes 238.520	38.880	Mtuberculosis 238.SED TTOEA	8.520	Kpneumoniae 238.SEQ	Minfluenzae 238.520	Bbrochiseptica 235.880A.C	Bparapartusais 215.8EQA.C.,	Bpertuseis 238.520	238.820	238.820	Bpseudomalle1 238.8EQ	Ngonorrhoese 238.820C.,	Neminigititdis 238.520C	Paeruginosa 235.520	Vcholerae 235.5EQ	Vantarocolitica 238.883
	Baubtilia Renthraci	Efacaelle	Llactis 235.SEQ	Lanonocyte	Saureus 215.SEQ	Smutans 2	Spneumoni	Spyogener	Mavium 2	Mcubercu	Ecoli 238.5EQ	Kpneumon	. Hinfluen	Bhrochie	Bparapar	Bpertuse.	Bompacia 235.520	Bmallei 218.520	Bpseudom	Ngonorrh	Nemin191	Paerugin	Vcholera	Yenterroc

Allgnment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	120	130	3.4	150	160	17	=	180	190	200	210	220	
Baubtilis 238.8EQ		SCICTO.	J. Mar. Cano	COLCTOS. AAT. GAGTGGCATTATA. G. GAAGC	T. T.	ATA	3				0	G G A	A 230
Banthracia 215.5EQ	•	OK.420	S.MT.GT.	CCA. ACG. AAT. OT. TCG C T	T	ATD.		5			2		A 230
8		A.A.C.	T. ATAGG.	A.A.CTTAINGG.T.TCTTC.G.		.C. G	AC.GA.T.GATAGOCA.A	MG GCA.		H	3	GG.	A 230
Llactis 238.SEQ C.	C	6	.CTCC.A. TMGAGT.	AGTTCA.G	TCA.G.GA		.C. CA. OTAA TA OCA.A.	8	A	• • • • • • • • • • • • • • • • • • • •	:	. :	A 227
Lachocytogenes 239.520	.0	C.A.C	T. AGROSS.	.C.A.CTT.AGT030.T.GCCSACGTGOGAAGCA	8	ogg	8	5			:		A 230
Saureus 235.520		₹.5	.CA AD AT TOT.	101G	G TG A	T	.CATA.C.GAACCA.	,	A	H	0	K	A 231
	λ	O-KOYO	3.MT.C	.ACA03.MT.CTOTCATA	TA		Th.0.ChhdOCh.T	88		C0.TCC.	:		G 223
Spneumoniae 235.520		ACAC	G.ANTAC	ACA00. AATACTOT C. CAC. T T A	TTA	ATG.	ATG. G.G AAG GCA.T.	8	:			K 0	A 224
OE OE		£50.0	O.Mr.CA-	0.CATO.AAT.CA-TOTCCA.GTA	TA		CA.G.GAAMGGCA.T.	8.6		•	.G. TOC	₹gg	A 224
Kavíum 238,880		CAC	G.C G	CAG.NG.GATOTOTC.CGTATGTGCGGGAG.TGC	T AT.	3000	COGNG. 1-	8	0		5	:	T 237
239.SEQ		C.000	0.0MTG	TOC.00C.	TAT.		COCHO	8		0	5		T 230
	TTCCOUNTCCCOUN	CCCAOTOTO-	ATTC-STCA	CACTATCATTA	ACTGAATOCA	TTOOKT	PATGAGG	CANCOCCO	CONCION	ACATOTANO	PACCOGRAG	NACCONTOTO-ATTC-OTCACASTCATTCATTAACCTENATCCATAGOTTAATGAGGGGAACGGGGGAACATCTAAGACGTGAAGACGGGGAAAAAAAA	£ 228
Kpneumoniae 238.8EQ G	0	5	Z			;	Ü			••••••			. 228
Hinfluensee 238.88Q A.	A	TADA	AGATGANG-AATCT	T	-		8	A	AA	TCOTAA		• • • • • • • • • • • • • • • • • • • •	. 232
Bbrochiseptics 238.5EQA:	••••••	30.8	Desenses!	.CG.CJA		8	ione				D. F.		. 224
Sparapertussis 238.850A		8.C	D	.03.CM	:	A		F	J	0	C0.1		. 223
Bpertuseis 235.920 A	A	8.C		8.88.8	A	cc-Aord	STG		•		0.7		. 223
_		-80.0	.C. CCTTGG.G	20.0		Š	.cc-ATGCA	8		• • • • • • • • • • • • • • • • • • • •	8.4		. 224
Bmallei 235.5EQ · G		0.001.	T T.	TOGOTCC.AG.	A	00	0	8			A	• • • • • • • • • • • • • • • • • • • •	. 225
Bpseudomallei 238.5EQ G		a.œT.	T T.	TOOOTCC.MG	:			8	A				. 225
Ngonorrhoese 218.5EQ G	• • • • • • • • • • • • • • • • • • • •	-00	10H	C., CTOTG., GC.A.GT.	.c	•	CJCBA	C					. 228
		CC	:	-TOTO. G C.A.GT.	•	AACTGAA.	. A.	4O	Α	C	0	• • • • • • • • • • • • • • • • • • • •	. 227
Paeruginosa 238.8EQ C	C7.	8cm	1 A-001	CCTA.QAA-CCTGGT.GT		GC.A	3C.A	A					. 228
Vcholerae 238.5EQ	•••••••••••							A				0	. 226
Yenterocolitica 238.SEAC		ี่ วี		3 TOCATOG.	T00A	OCATOCA.	MACA			•	•		. 228

FIG. 2B-2

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	-	3 5		7 6		; ;	3 5	5 5	200	364		, ,	9 0	900		7	278	278	1 .	2 1	187	284	283	282	281	286
										C-C ANCHOROTHANCOCATIONATORS COTT G. TIOT. T. TIC. COOPTIONS THEN THE PROPERTY OF								-		# # # #					3 3 3 3 5 5	
	ç									֓֞֝֜֝֟֝֟֝֓֟֝֟֝֓֓֓֟ ֓֓֓֞֓֓֞֓֞֓֓֓֞֞֓֓֞֞֓֓֞֞֞֓֓֞	֓֞֜֜֜֜֜֜֜֜֜֜֜֜֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓		ŧ			j	j	5				!				
- 82	ر د د				É	î				į	: }	43.6	ŧ				T.T.				T.A.T.	A.C	A.C.	T T.	0	
										TCATAT	44.40								E							
-	Traceout	TACCAC	TACCAC	OR DO GAR		TACCAL	TEACTE	TAGGAC	TAGGAC	TOOCE	000000															
	TOOL	COPI	CCCTT	3)	COUL	COOL	COOTT	COCT	00												*****	1 1 1 1 1 1 1 1		
270		1		T. T.	į	T. C. L.	ľ	CICTIC	رير. بر ريد	T. T. TOC	7.7.70	0AGGAGCCCAGAGCCTG		OT ACT			table to the second		2			T- TA G	TTA.G	- T. T	13.20	Α
~	20	20	4	4	•	£	7 111	į	E	G. Targ	0	200000		4		•	• •				•	# (E	Ë	:
	3	5	5	5	1	อี	5			C. 001	Ç.	7														
			34	386	AAC		200	3	34	SACAGO	OCCUPAC			ACT. ACTOR CONTRACTOR				1	7 7 7 7 7					-	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
	5000	GOCCAAACCA AG TROOCT T GCOTHOTEGCEC		GOOCAAACCAA THOCTT T COSTITUTIONS	COCCAAACCA A TTCCTT T CCCTTTATATACOAC	GCCCAAACCACA. TTGCTMGT. GGGTMGTAGGAC	GOOCAAACCAGA.T.TTT.C.CTGGOTTOTAGGAC.		ACCA-66A	LY TOCKT	2000		1	,				3) 		
				į	,	1	į			NACCO.	NACCK.		****		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				1							
:	CGA. C-AC GA-7CA	ACAACATA	A-202-	C.AA-GAA	ACAACA	A-AGA-	-450-45. 48-	A-GM	A-00-4	COCC	2000		****			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				7				; ; ; ;		
36	Ų.	-AC . A	NCGA-3GA	₹.0	- YC Y	-ACQA-AGA	3	ACCA-GAA-	XCC	Α	₹.:>8	4889				717	- 210	5	-AT	TAT	,	֓֞֜֜֜֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓	;		Ė	
250	8											AGENGCOCONOCONA-COCO										•		:		:::::::::::::::::::::::::::::::::::::::
		:	:			<i>-</i> :	:	:		E	f	DOCCOOK	:	:	f	e	Ē	ŀ	t	8	f	E	•		:	::::
240	2	2	g	£	g	Ė		#	Ė	5	:	¥		5	3	100	:	:	4	1		:	; ;	{	; ;	::::
230. 240	C.	T.C T TO.	TCT0	MCT.OTA	£	#C#	A.C 0 T.	A.C 0 TT	.C G T	T.T QT.	H	CONCANTICOCC	•	4.9			3	***************************************	***************************************						3	
				2	S. SEOA	H	4		•	E+	T 023.	σ			8.5EQ.	9.680.	0		•	025	CAS	S. S. S.				38.5EQ
	Baubtilia 238.SEQ	Banthracis 238.SEQ	Efacaelis 238.8EQ	. 850	Lmonocytogenes 235.5EQATCTTG	. SEQ	. 8EQ	Spneumoniae 238.SEQ	Spyogenes 238.520	820	Mtuberculosis 235.520 T.TG.A	ន្ត	Koneumoniae 235.520	Hinfluenzae 235.SEQ	Bbrochiseptics 238.820	Sperapertussis 238.520GAA	Bpertuseis 235.820	8.8E0	SEO	Bpseudomalle 238.580	Monorrhogge 238.8PO	Newiniciteitelia 218 880	310		30.00	Menterocottetca 215.5EQ
	:1110 3	wacte	nelis 2	Llactis 235.5EQ	cytoge	Saureus 235.6EQ	Smutans 235.8EQ	monia	Jenes 2	Mavium 238.8EQ	rculos	Ecolf 138.520	montas	Luenzae	thisept	Ipertue	susota	Bcepacia 235.5EQ	Bralle 238.5EQ	domall	rzhona	statete				Locoti
	Baubt	Banth	Eface	Llact	Laond	Saure	Smuts	Spner	Spyog	Kavi	Mtube	Eco11	Kone	Hinel	abroc	Bpere	Bpert	Bcepa	Brasil	Boom	Moone	Nemir				X 6.11 C

FIG. 28~

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

		425 426 426 426 426 426 426 426 426 426 426	
	370	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	•
		TOGAL-O TAGAL-O TAGAL-	
	- 69 -	CT C. A. C. C C. A. C. C C. A. C. C. C. A. C. C. A. C. C. C. A. C.	
	. မှိ-	### C. TOGTTCTCT. — C TOGAT—CO. ###################################	
	340	A A T. OT. C. TOTTCTCT. —C. TOGNT—CC. C. A A G. G. G. TOCTCTCT. —T. ATGTAT—CC. C. A T. G. G. TOCTCTT. —T. ATGTAT—CC. C. A T. G. G. TAGOCTT. —C. ACUGNAT—CC. C. A A T. A T. TOCTCTT. —C. ACUGNAT—CC. C. A A G. T. TTG. TOCTTTCCT. —T. TOGNT—CC. C. A A G. T. TTTG. T. CA. TTG. G. —CAACACAN—CC. C. A A T. TTT TAGT. TTG. —T. ACCATAN—CC. C. CACACAN—CC. C. A G. G. G. G. COCOG. ACTCCCT. ATACAN—CC. C. CACACAN—CC. C. CACACAN—CC. C. CACACAN—CC. C. CACACAN—CC. A G. G. G. G. COCOG. ACTCCCT. ATACAN—CC. C. CACACAN—CC. C. CACACAN—CC. C. CACACAN—CC. A G. G. G. G. COCOG. ACTCCCT. ATACAN—CC. A T. AT. T. G. G. TOCAG. GTG. CACAC. AG. GTAA—CA. T. T. G. G. TOCAG. GTG. CACAC. AG. GTAA—CA. T. T. G. G. TOCAG. GTG. CACAC. AG. GTAA—CA. T. T. G. G. TOCAG. GTG. CACAC. AG. GTAA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CA. T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CAL T. TG. A. CACAC. AG. TOTA—CA. T. T. G. G. CACTT. TOCAG.—TGA. ATC————————————————————————————————————	
-	330		
-	320	Benthaeris 138.5EQ - GANTHOLMORACA - G AC GONDA	
-	310	0 - 0-TT-	
-	ន្តា	MCCA —— G AT GAG MCCA —— G AT GAG MCCA —— TA T GCG MCCA —— TA AT GCG MCCA —— TA GCG M	
L			
I	2 2	AGAMCA. AGAMCA. AGAMA.	
	Chorenous	Benthracia 138.520 - CACTYCOLMA Efacate 135.520 - CACTYCOLMA Liactis 235.520 - CACTYCOLMA Liactis 235.520 - CACTYCOLMA Saureus 235.520 - CACTACALA Saureus 235.520 - CACTACALA Saureus 235.520 - CACTACALA Spreamentae 235.520 - CACTACALA Spreamentae 235.520 - CACTACALA Spreamentae 235.520 - CACTACALA Reberculosis 235.520 - CACTACALA Reberculosis 235.520 - CACTACALA Reberculosis 235.520 - CACTACALA Reperculosis 235.520 - CACTACALA Reperculosis 235.520 - CACTACALA Spreamentae 235.520 - CACTACALA Spreamentae 235.520 - CACTACALA Reperculosis 235.520 - CACTACACALA Reperculosis 235.520 - CACTACACACALA Reperculosis 235.520 - CACTACACACACACACACACACACACACACACACACACA	
F		35.529 	
	738.SE2 1	18 235.587	
	Baubeilis 235.SEO	Banthracia 138.5EQ Liactis 218.5EQ Liactis 218.5EQ Liactis 218.5EQ Emuraus 218.5EQ Emuraus 218.5EQ Spyogenes 218.5EQ Spyogenes 218.5EQ Mcuberculouis 218.5EQ Mcuberculouis 218.5EQ Mcuberculouis 218.5EQ Mcuberculouis 218.5EQ Minfluenzes 218.5EQ Bertuseis 218.5EQ Bertuseis 218.5EQ Bertuseis 218.5EQ Remallei 218.5EQ Remallei 218.5EQ Womorrhoese 218.5EQ Weminigittidis 218.5 Bearuginosa 218.5EQ Veholerse 218.5EQ Veholerse 218.5EQ	

FIG. 28-4

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	380	390	904	410	420	430	440	650	460	410	480	- 68	
Baubtilis 235.52Q	٠,٠	₹	8		¥		E					A A	
Banthracis 238.5EQ	٠٠٠٠٠	7. E.	U	4	į				•	H			244
Efacaelis 235.8EQ	.AA.		8		8				••••••	F • • • • • • • • • • • • • • • • • • •		.hhhhhhhhhh.	345
Llactis 238.8EQ		Z	78	λ	ς Σ		e e			•	::::	G.AA . A. T	537
Laonocytogenes 238.820.AAAT.C0cATCATCATCATCATCATC	120.A.	MT.C.	8	A	2		t						233
Saureus 235.520	Ŋ	X.7.C.	. NO NA . T.C G C A	Α	7.7.		TC. T.			•			
Smutens 239.8EQ		A.	.coc.	AC.	CACTCCC		Ċ				: :::::		9 6
Spneumoniee 238.550		₩		4	35		ť						673
Spyogenes 238.5EQ	AC. &	7	0		ų Į	e	ţ				·····		230
Mavium 238.820	G.C	λ	8	U	6	r	E.				····	19C. 9	531
Mtuberculosis 215.5200.C	o.c.	A 8	8.8	U	Ş		8						593
Ecoli 235.5EQ	achechecata	OTATOCHOL	CTGAATATOOC	COCHOCAN	CETCCARG	2CTABATA					3	OCACHOTOTRACTOR TRANSPORTED CANCELLO CANCELLA BATTET TO THE ARTEST TO TH	587
Kpneumoniae 235.520							5		,	- CHARLES	MANAGE CO.	COOCUMOOOCHOL	
Minfluenzae 238.8EQ			T0.A.	U			F		•		•		669
Bhrochiseptica 238.8	081	1	o.				1	•					\$08.
Sparapartusais 238.880	Q.	4	O				3					X	
Spertuseis 238.SEQ	***************************************	2	O				1	•			,		492
Bospacia 235.880	•	2	9				į	•	•	W		···	492
Brallei 238.820	X		0						•	• • • • • • • • • • • • • • • • • • • •		07	497
Bpseudomalled 238.SEQ	g	•	0				į.	•	•				497
Monorrhoese 218.920		7						•	•	•			497
Neminigititals 238.580. G. A.	BO 0	1			•			•	••••••	•••••••	• • • • • • • • • • • • • • • • • • • •	000 · · · · · · · · · · · · · · · · · ·	200
Pasruginosa 238.830	Α	1	U						•	•••••••		609	499
Vcholerae 238.8E0						•		•		• • • • • • • • • • • • • • • • • • • •		990	690
Yenterocolitica 218.820	SEO	9			•			•	••••••		• • • • • • • • • • • • • • • • • • • •	.T.T 487	487

FIG. 2B-5

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	- 200	510	520	530	540	550	260		570	580	590	- 009	
Bsubtilis 238.880	. O.		.0.0.	5	6.5	A.C.	B	0	0	9	8	18	١
Banthracis 235.5EQ	0		0.0	. AT G		A.C.,-,.,176,,6,,6,,AAC.G.,,G.,,C-CCT., G	2	o	0	o	8		
Efacaells 238.SEQ			a.c	£5		A	2	0	0	g	8	TG-CAT	9
Llactie 238.880	TC	•	0.0	ATC.		A	5	O	9	g	8	A - AT	
Lmonocytogenes 238.5EQCTT.	P		0.C.	.TT.		A	á	o	0	9	8		Ì
Saureus 238.SEQ			0.7.	5	9.7	A	2	0	0	o S	8	T A.T.	•
Smutens 238.880	g.	:	0.3	A	G. C. Th. C. C. C. C. Th. C. Ch. C. Ch. C. Ch. C. Ch. C. Ch. C. Ch. Ch	A	3	0	Q	9	8	T3-003	9
Spneumoniae 235.8EQ			0.C.	g			3	0	4	ÿ	8	A - AT	9
Spyogenes 218.5EQ	-	•	a.c.	2		A	3	9	4b	g Q	8	A - AT	
Mavium 238.850	4.4		0.0		6.c		8	0	A.GA	Ľ	G.C. 000	20.00	809
Mtuberculosis 238:520G.T	0			Q. 0.1.	c Trocher	COCHOCHOC. T.	ę	Ü	¥ 0. ¥	Ę	0.00		
Ecoli 235.SEQ	GAMMON	CCTGALACK	COTOLACOLA	PACCAGTOO	GANANGNACCTGNACCOTGTACNGCAGTGGGACCTCTTTTATGGGGGTACTGGGTACTTTTTTATAGGGGGGGGGG	TATOX	STOACTO	GENCCITIES	TATAMO	OTC POC	ACTURATE		_
Kpmeumoniae 218.SEQ		•	•	•	£ 0								
Minfluenzae 235.880	E				GAGGC								
Bbrochiseptica 218.8EQTA.G.AC.	Q T 1		A.G.A				O				·		
Bparapartuseis 238.8EQ T	D T D		A.G.A.				0				Ü	-4	
Bpertussie 238,520	T		A.G.A			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	O				ú	-	
Bospacia 235.880			C.O.D.	AC		A.G	0				8	Ą	
Bmallei 218.8EQ			C.O.A	AC	.C. C. G.A		Ö				8	4	
Bpseudomalle1 238.SEQTT	Z Z		. C.0.2.	AC	AA	000	O		2		3	<u>ا</u>	
Ngonorrhoese 238.880C.			TGA.O.A.	Ä	ı		•			•	,		
Neminigititidis 238.580 C			TCA. C.A.	, i	TGA.G.A.	T					; ;		
Paeruginosa 235.8EQ	f		A.O.		.A.GA.G.	AT.D							
Vcholerae 215.8EQ	F				A	- D	£				:		
Yanterocolitics 238.870C.	200				ر	i				•	•		
										:::::::::::::::::::::::::::::::::::::::			603

FIG. 28-6

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

			-	-						
	0 -	620	630	040	650	999	670	680	069	
Baubtilis 235.5EQ	G.AG.7	C		ă		The state of the s			١	163
Banthracis 238.880		Ü		12.0		ACOT TOTA				:
Efacaelis 238.830		Ų	0	į.		PHGT 144			6/ DE	3 2
Llactis 238.SEQ		Ş	-	£ 0				É		2
Lmonocytogenes 238.52QG.G			-A.CTC	Q						: :
Saureus 218.SEQ	G.A.T.A.T.T.	F. 7		72.0	000000000000000000000000000000000000000		•	· · · · · · · · · · · · · · · · · · ·	ָּיִנְיִינִינְיִינְיִינְיִינְיִינְיִינְיִ	
Smutana 238.5EQ	OTT G MC	Ş	J. C. C. L. C. C. L. C.	47						3 5
Spneumoniae 235.5EQ	940	9	E	G. TA	*********	T. AT. T ACC		,	· ·	, t
Spyogenes 218.880		ÿ	-	TA	077 G A	T. AT. T. G	•	,		
Mavium 216.880	C 1000	F .	CC	QTA		0.07.7	O	400		ָר אַ מי
Mtuberculosis 235.5EQ			cco	GTA.	Neuberculosis 235.580CTd.GTCCGAccescaceconing and a grant G.	7. 70. 0. 1	U	8		200
Ecoli 235.SEQ	OTTAN-COGMATIN	20-00000	CCANAGCCANACCC	MOTOTEMOCOG	OTTAN-COGNESSIANICCANGGGANGCGANGCTTANCCGGGGTTA	- AGTTGC AGGGTA	PGACCCGA			200
Koneumoniae 238.880	£1			£-						
Hinfluenzae 238.550			•	ŀ	7. AAT	4			, C C	<u></u>
Bbrochiseptice 238.8EQ		DA	1. TCA.A.N.G	8. TA	Bbrochiseptice 235.880C	ני		·		
Bperapertuseis 235.820C		Q4	1. TCA.A.N.G.	8	20 - 10 - 10 - 10 - 10 - 10 - 10 - 10 -	£ .				
Bpertuesis 235.SEO	C2	P A 0	1. TC. A. N. O.	8	AGTCA.A.N.GCGTA.		•		· · · · · · · · · · · · · · · · · · ·	9 9
Boopacia 238.880	0	0		8	9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9		:	; ; ;) () () (
Bmallei 238.880		0.0		8		5	•		700 J	1
Bpseudomallel 218.5EQ C	C		CGTCGTATA	8	***************************************					ב ב ב
Ngonorrhoese 239.8EQ Cd	C	9		TA	A. Grant and an annual section of the section o			đ	•	200
Neminigitatis 238.5EQC		D	TA.G	TA		20 C		5		, y
Paeruginosa 235.SEQ	C	DT	*.TGTCGTAT.	TA		£ .0		U		6.5
Vcholerae 238.850			D.DT	F		•		e		
Yenterocolitica 238.5Eg		•	T	H		Α				9 Y
								••••••		2

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

•	710	710	720	730	740	750	760	0,77		780	790	800	810	
Baubtilis 235.520	J. 7. C.	2	Q. A.	 	0.36.0		6.00.00	8	.O. A.	F	0	£		E
Banthracis 235.820	2.5	Q.F.	Q.AC	:	c8c.c	:	0.00.0	8	G. 0C. G0G0.A. CA TT GT	F	0			.:.8
Efacaelis 235.8EQ	8	:	8c		cgc	:	G.0C.G	8	.G.CC.GOGG.A.CA	•	.TT A. G	1		.T 856
Llactis 238.8EQ	9	:	.06		ACCAC.C.	:	O. GTTT.		G. GTT	E	.TT A.G T		:	7.8
Lamonocytogenes 23S.SEQAAA.	λ	:	.TTT		.c6c.c.	:	.c.86.6	8	G.0C.G0GG.A.CATTGTT	£	9			.t.
. Saureus 235.8EQ	5.5	:	G.A	:		:	.0.0	ACA	G.GACAG.A.CA	E	gT.	.T	:	
Smutana 235.5EQ	8	:	.AGC	•	MODAC.C	:	0.0777	••••••	G.GTTT	#	TTA.O TT		:	
· Spneumoniee 238,SEQ	8			:	.XXXX.C.	:	G. OCTT	:	G.A.CA.	:	.TTA.GTT.		:	.T.
Spyogenes 218.550	5	:			AGGC.C	:	.0.0CTT	•	G. OCTT	#	A.G	# · · · · · · · · · · · · · · · · · · ·	:	T 850
MAVIUM 235.880	0	:		c.	GT.GG.C.GGGCG.A.	0	C. GQ	8	. G.A.		.1.00.1.	TC. T.	:::::::::::::::::::::::::::::::::::::::	6 ::
Mtuberculosis 235.5EQ G CGC.	0				CT.GG.C.GGGCG.A	0	C.GO	8	A.D.			TOC. T	:::::::::::::::::::::::::::::::::::::::	6 ::
	CLOOTTONOOTTO	OOTTOOCTUA	gerackctractogaggecgaacceactaatgaaaaattagcgaaggatgactgoggggaaaaggccaatgaaagccaatcaaaccgagagatagtgogtgtccc	ACCIDITATION	CCACTAATGE	A P	NATTAGOGG	ATCACTION	1000001700	SAMOOC	SATCOMO	COCCACATAO	croorie	800
Kpneumoniae 135,820											•	• • • • • • • • • • • • • • • • • • • •	•	8 :::
Hinfluenzae 136.580		•		•									:	823
. Bbrochiseptica 238.8Eq		90				٠	c0		A.A		T	T. A T.T A	:	.T. 807
Bparapartussis 238.580CAC	•	ck	gre					8			r	.T. AT.T A	:	.T. 808
Spertuseis 238.520	90		976			•		8	A.A	•	TAT.TA		:	.T. 808
Beepacia 235.580	A	8	5		cc		00	8	A.A		.T. A T A	.T		H
Emalle: 238.5EQ	A	8	5			:	0	8	A.A		TA	.T. A.		T. 81
Epseudomallei 238.5EQAOC	y	:	8	E+	CC	:		8	A.A.		A	T. A T A T.		T 81
Ngonorrhoese 238.550		88	£50			Ü		8	A.D.		.T. A	<u>g</u>		81
Neminigititals 236.820			8				8	8	A.A		T A T.			:
Paeruginosa 238.880	? ······	Α				:	000		. A.C. A.		£	.a.1c		T. 807
Vcholerae 235.880		Α	9		• • • • • • • • • • • • • • • • • • • •	:		:	A	:		2	:	80
Yenterocolitica 238.880		•	•			:				:	•			:

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	820	830	840	058	860	870	.088	068	006	ote	920	930
Baubtilia 235.5EQ	TAGC	: 1	₽.4	OCTA AA OT. A-0. OTCTT A	~	A - T	AT. ACTAC.CTCAGG.		G.ATTC.OT.	U	G. AAT. C A	֟֝֞֟֝֟֝֟֝֟֝֟֝֟ ֓֓֞֞֞֞֓֞֞֞֓֓֞֓֞֞֩֞֓֓֓֓֞֩֞֩֞֩֞֞֩֞֩֞֩֞֩֞֞֩
Banthracis 235.5EQ	Dec	8	T10	. TAN TOTANG. OTCTTA.	A	AT ACTA.	:		O.ATTC.OT.	Ü	GAATCA 978	A 97
Efacaelis 238.5EQ	TAC	S. S.		O-AATTGAG: ATGAT A.	Α	4	ACTAC.C	TACTAC.CT0G		:	GATTC.TA 971	A 973
Llactie 238.SEQ	TMC	- :	MATO	GCTAGMAIGIAAGIGIATTA	Α		OTGA		T.TC.GAT.	:	G. TANTAC CA 96(5
Lanchocytogenes 235.5EQTACC	TAGC	8	₹	AGT.AAG.GTCATA	A		ACTAC.C	C.CTT86	O.ATTC.GAT.	J	G ATOT. C A 978	37
Saureus 238.520	TNOC	octa	Z-72	.A TO-A.GTATTA.	Α	TACGA.	ACCAC.C	c. 818	G.ATTC.CA	O	0AATTT 97	T 97
Smutans 238,520	TXC		7 gross.	CCTA G GTCGC-GAG. CTCTT A.	A	4.£	-4.ATTGA	5	T.TC.GAT.	J	. GA.C G A 963	A 96.
Spneumoniae 215.SEQ	2000		ACATT	OCTA G ACATT. CAG TCTT A.	A	-T. GTGA.				:	GAAT 963	A 96
Spyogenes 238.820	TAGC	•	AT. TT.	.OCIAGAT.ITADICICITA.	A	T OTCB.	:	g	T.TC.GMT.	Ü	GAACCAA 965	AA 96
Mav1um 235.8E0	700	•	3.T. C.TX	.OCAG.TC.TOG C.AA.		ro-AT	COMT C.	:	.TGOTC.OC		0TG.TG.AA-A	.AA-A 104
Mtuberculosis 23S.SEQTGC			3.T. C. TO	0Ch0.TC.103C.0hhT0-ATCATC.CA.TA031001C.GC.	Α	rG-AT	.ccarc.(2A. TAGG	.10orc.oc		CGTG.TG.AA-A 106	.A-A-106
Ecoli 235.5EQ	QUACCENTITY		1001-0-N	30Thg030CT00T-0-AATTCATCTC00303GTAGAC-ACT0TTTC93CAAG333TC-ATC0G4ACTTACCAACGAACT	POOGTNGAGC-	ACTOTATOOS	CAAGGGGGTC.	-ATCCCCACTTA	CCAACCCAATG		ACTGCGATACOGGAGATGTT-	TOTT- 932
Kpneumoniae 235.530	•••••••							•				93(
Hinfluenzae 238.880			7.4-1-6	AT.AGTGACTT	•		C			Α	AAGA	A 938
Bbrochiseptica 238.520A			.TM.	A T. TT. CTO. A.	•	ATT			A ATO.	Ü	3. G. S	.AC 920
Bperapertussis 238,820 A	A		AT.	A T. TT. CTG. A	•	ATT.		8	AATG		5.F.	.T.CA.G.AC 92
Sportussis 238.820	A	:	AT.	t	,	ATT.	:	AArd.	AATO.		5.5.	T.CA.Q.AC 92
Bcepacia 218.820	A		R	TCTCA.C. T		CATTTG.	:	TTG.AGACCGATA.	000			A0C-A 92(
Emallet 235.620	A		¥	.TOTOS.C.T	•	CMTTTG.	:	T. TO. AGA.	000. ATA.	o	A-00-A	3C-A 92
Bpseudomalle1 238.58QA	y	£	5	TCTCA.C.T	•	C.TTG.	:	.TTO. ACA	830MEA.	C	A G C-A	3C-A 92
Ngenerrhoese 218.5EQA	y	:		ACCACTGAT		ATT.	:	TTG.A	AIG.	5	ATCA.	ATCA. G. G-T 93
Neminigititdie 238.880A	A	J.	AC.	ACCA CTCAT.	A	ATT.	:	TTG.A	AIG.	***************************************	ATCA.	ATCA.0G-T 93
Paeruginosa 238.SEQ	•	•	A	AT-ACICIG.		•	£ .		Α		COG.	CAGA. G 000 92
Vcholerse 238.880	••••••	• • • • • • • • • • • • • • • • • • • •	04-0	0-A-CGAAT. CTA. T.		XXT	4		E	Ü	A. T.	A. TA. G. AC. A 920
Yanterocolitica 235.880	Q		0	C.	, , , , , , , , ,		E			U		6
•											•	

FIG. 2B-10

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Benbtille 238.8EQ TI Benthracie 238.8EQ TI				-	2 -	086	?.	1010	0707	1030	1040	1050	_
	TC. TT T G	T A A G.		A-80.A	С			OTE 4	l	ļ		1	
	TC.TTAT.O			A. C. A.	c				:				7 7
	TATCTO	A		8	•		· · · · · · · · · · · · · · · · · · ·		:		Tr. Tr.	:	9601
Llactis 238.880 T	TOTT C T G			₹ 80 - ₹	e	4		- 1717. C	:			:	6801
Lanonocytogenes 238.820TA.TTG.	b.TT.	4	:	¥-8		4			:	:	4 l		7801
Saureus 238.SEQ	A.TT T 0			G-T-G. A	•	A			:	:		:	9601
Smutens 238.SEQ	GMCC70		:	8 -4	G				:			:	9601
Spneumoniae 238.5EQ T	703C1G			8	•	4	•		:	:		:	1087
	TART. C. T. G. T P.	•		4 E			•		:	:	Ort.	:	1083
	0.0710.0		: :	A 50. 54		¢		A.A.C.	:		4	:	1083.
Mtuberculosis 23S.5EQ -G.Gr.,C.,TG.G	0.01.010.0	:	•	4 9	-				-		TAGTC . AG.		091
Ecoli 238.SEQ	ATCACCOCACACA		- aconconc	CTCAACACC	CANAL DAGE					CAGIC AA	Caste. A		. 179
Xpneumoniae 235.SEQ .	J								377775	TOTAL STATE OF	SCORMOOD .		1050
Hinfluensae 238.520	CFAC			Ö			•	-		: 4	₹	:	1048
ä	0.11	A.C.	U U-fg	Ų			•			•	H		5501
Sparapertussis 238.820.0.TT	a. tt		ฮ	0.0-40			•		֝֟֝֝֝֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֡֓֓֡֓֓֓֓֓֡֓֜֡֓֡֓֡֓֡֓֡֓֡֓֡֡֡֡֡֡		X	:	1038
Bpertussis 238.520	O.TT		5	υυ- 5					֝֝֟֝֝֝֟֝֝֝֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֡֓֓֓֓֓֓֡֓֡֓֡֓֡		* · · · · · · · · · · · · · · · · · · ·		6001
•	D	•	8	5				A 474 A. O.	,		4 1		6001
Emallei 238.520 .	9	λτ	6					C. A Arro -C	ų				
Bpseudomailei 235.SEQG	0	AT	5						,	•			600
Ngonorrhoese 238.520 fc.T	D		,	ر ا		C			:		A	A.A	1043
Neminigititdia 238.880TC.T	D. 3		Ę	Ü					:		₹		1051
Paeruginosa 238.820	.d			4					:		: '	:	1050
Vcholerae 238.830 -	1C			U			- - - -						1040
Ŋ												:	1035
								:::::::::::::::::::::::::::::::::::::::	•		¥		1049

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1060	1070	080	1080	1100	1110	1120	1130	1140	150	1160	
Baubtills 238.520	λ			6		3	GACT. C. A. C.		A G A	Į	- Acmes	
Banthracis 238.8EQ	A.T		9	5		ð	O.C.		טעט אַע פּי	Ų	CHAIR!	1213
8	A.T			:		3		٧.	4		T. Profits	
Llactis 238.880	A.T A.			8	Α	Aauc	0	λ	C A C ATT.		TT -ATTO	
33.820	λ			QT		3	•	λ	· · · · · · · · · · · · · · · · · · ·		A.TAT.	
Saureus 238.500	A.T.			g	A						TATTO	770 1211
	A.TA.	P	TC	•		:	GACCACGAATTT.	λ	K	£	7ATC.	JC. 1200
Spheumoniae 236.5EQ	A.T			5		Α		CAC	AATTT.		TATA.	•
2	A.T A.			5		AGac		λ	C. A. GITT.	1	TAT.	T. 1 1202
Mavium 238.880	AG	• • • • • • • • • • • • • • • • • • • •		8		3 · · · · ·		C	0.0	9	CACAT	CAT 1279
Mtuberculosis 236.620 AG	λ		8	5		AG	C		00	9	T COLO	CAT 1298
Ecolt 238.SEQ	accuatanton	SOCIETA GALACK	CCAT-CATTER	ANGRANGOSTA	ADAGCTCACT	SOTOGAGIC	CTTAANACHCCAT-CATTTAANAAANGOTAATAGCTCACTGGTCGAGTCGGCCGGAACATGTAACGGGGCTAAACCAT-GCACGAAGCTGGGG-CAGC	KATOTALCO	SOCTANACCA	ACCOCCA.	DCTCCCC.	
Kpaeumoniae 235.8EQ		•			••••••							•
Hinfluenzae 235.8EQ		• • • • • • • • • • • • • • • • • • • •						•		:	-	
Bhrochiseptica 238.880.TG		•	00					• • • • • • • • • • • • • • • • • • • •		:	1.55	
Sparapertussis 238,880.Td			CC		:		4	••••••	G.GA-A	A-A	1.19-	T.T 1154
Spertuseis 238.580	0	•••••••••••••••••••••••••••••••••••••••	00			.A			G.GA-A.	A-A.	Y	-OT.T 1154
Bospacia 338.580		• • • • • • • • • • • • • • • • • • • •	0-0			A	£		G.TATA-	ATA-	-AT.	Nr 1160
Brailet 238.820			00	•		A		••••••	GATA-	ATA-	1	AT. 1162
Spaeudomallel 235.5EQ .T			0-0	• • • • • • • • • • • • • • • • • • • •		. A T		•	OATA-	ATA-	7	
Ngonorroces 218.880	••••••					•			C. ATC. ATA.	ATA		VT 1169
Neminigations 338.880								***************************************	C. ATC.ATA.	ATA		AT. 1168
Pagruginosa 238.500			0-0		•	· · · · · · · · · · · · · · · · · · ·		••••••	c	ð	i	T 1156
Venoterae 238.850					• • • • • • • • • • • • • • • • • • • •	AA			OAEA.	EA.		AT 1152
Manterocollitica 238.880								•••••••	• • • • • • • • • • • • • • • • • • • •		1	1166

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	-	ŀ		-	ľ										
	1170		1180	1190	1200		1210	1220	1230	1240	1250	1260	1270	1280	0
Baubtille 215.SEQ	TT.T.	8-8	5	1		-A.O.G		ט טיין איני טיין טיין טיין טיין טיין טיין טיין ט	•	1	1				.
Banthracis 238.520	ATAC. MIG		-A.C.O.			-A G A A				:		5 6			0 132
Efacaelis 215.5EQ	CC.A.	4	-CIC. A. TA OTTONOT	Α		-A.0.0.0.		22.00	m 550			.c.orgghdh		\$	6 132
Llactis 235.520	£.	Ę	TA. T TAT-CAA-T	Α		.TA.006.GA.		5	ą	4	· ·		* 6		0 132(
Lmonocytogenes 218.SEQAC.T.T.TAA	20.C.T.T.	T.A.	1.0.Toor.			-A.9.6.0.	:	TCMG C. CA. M.C. G.	u Q	£ 0,5	-	;	5 ·	151 D Milwa	itt b
Saureus 235.SEQ	4-C.4	4	-Cur.	λ		-A.Q.Q.QT.	:	CATG. TC. A. ACATG	ACATO				٠ 	:	0 1325
Smutans 238.5EQ	CTTA.90	2.9.0	A	A		Moro.oca.	:	ව ්	SSX	TCCAT			٠ • •	.	6 1322
Spneumoniae 135.520	CTT.AXD	AX	7-Y-1	λ		ATOTOTOR		υ γ		4000		; ;	5		
Spyogenes 235.880	5	DY-YO	cicun-voia.ca-7	A.		-ATOTOTEAA		t	:	#(J550)	•		æ		٠
. Mavium 235.880	104.1.	TAC. G	TCAT. T. TAC. GTOCH, OT	Q	Ü	Į.			:			c.grggAGA.A.	٠ 	4	Ġ
Mtuberculosis 235.550 CO.C.TCT/GTGG.GT. GTA. G. C.C C mt 2. C.	CCC.7.	5	700	1	Ċ	į.				61605.GAA.GGGGG.		¥.00.		₹	0 1397
Ecolf 238.5EQ	GCC-1	Ş	OTTOTION	TACOCK-A	CONTRACTOR					Grade.gAdgggg	Ą	A.G G.	DB	₹	0 1416
Kpneumoniae 238.5EQ									מאויאויאויאויי	-A	recentra	TOTOTO	TACCAT	Moccocy	70MA 1284
Minfluenzae 235.620	NON. 7 7C.	7	'n		AUDO . D	8			: : :			:::::::::::::::::::::::::::::::::::::::	: : : : : :		:
Bbrochiseptica 238.820.CMcTr. IN	D. C.C.T.	á	5	4			U	£	:	***************************************	:::::::::::::::::::::::::::::::::::::::			Y	:
Bparapertussis 238, SEQ. CACTT, TA	S. Cocra.	ž	5	4			Ü		3 8		: : : : : :				1265
Bpertussis 218.5EQ	CACTT. TA	គ្គ	5.5	Α.			ú			• • • • • • • • • • • • • • • • • • • •		9	66		1270
Bompacie 238.820	5	¥.+	.T ACTTACACAT	A.			Ü	8 Y Y	8			B	; ;	: :	127
Brallet 218.580	:	4	OCTA C. C. CAT	A	Ü		υ.	. M. A. B.	8	0		9 0	: :	; • •	127
Bpseudomallet 218.520 CCTAC.C.CAT.	8	គ	C.C.CG	A	ن 			.corA.A	A.8.	9		9	c		1197
Neminical Particles and Supplemental Company of the	8 8	¥ :	5			G TGA.		C.TA.AQ	A0			J	U		1283
Pastudinose 218.880	- T. C T.	*	20-CM			0TGA.	:	.C.T A.AQ.	λ0		T				1282
Vcholerae 238.880 Ar.TT. AGA. A.	AT THE	9						. Agr A A. Ct	۲.		:::::::::::::::::::::::::::::::::::::::		9	G	1272
Yenterocolitica 218.SECHRAN-MRN-MN	N-Magaron:	- KOL	Paramanananan D	C NOW	:		:	.A. TCA T.	F (• • • • • • • • • • • • • • • • • • • •	::::::	o	0	0	1267
		i					:		5			•		F	1284

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

			ŀ											
		1300	1310	1320		1330	1340	1350	1360	1370	1380	1390	1400	
Baubtilia 238.580	.T. T.C.A.	A. T.C. T	A.T.C.T.	8	NG. G.C. C. C.	E U	۱,	100	$\left\{ \right.$		15	ļ		•
~	.TTC.A.	A.T.C.T	E	100 00 00 CO	0.0					•		:	2	7007
ន្ត	T. T. TC. A	1 1		0000.0.000.00	9.0			į S			\$	-		200
Llactis 238.520	_	-	+	.TTTA.003., G.C.03., C.00.T	0.c.0	.a. 8.1	6	5			Ş			7433
33.SE	-	A. TAT. T T	F	Mag a.c	0.c.8	500	E	:		ט טע	ų	o og		1440
Saureus 235.5EQ	TC.A	ATT		A. A.O.	6.0.0	.ATTTTA.AGGG.C.CGC.CT.TT.	:	:		e	\$	C		
	.T. T.TC.A.	J T.	A	AA.0006.C.00C.0C.T	G.C.8	.c.8.7.			ď	A A T T	v	C		428
ន	. T. T. TC. A.	T T		.A.00G.	9.c.8.	.TTTA.036G.C.03C.0C.T	E		Α	A A	ý		• • •	1432
Spyogenes 215.SEQ	T.T.TC.A.	T T		.A.000.	G.C. 89.	.TTTA. 033G.C. 03C. 00.TT00A.	E.	:	4	.AA		C		1434
Mavium 235.520					0.00.0	 8		QG			ų			3
Mtuberculosis 238.820		Α	:::::	8	9.8.0	8 		808			ų	c		755
Ecol. 218.589 AACOCCTCCCCAAAACCAACATTCATCAACCTTAATCGGGGCAAGCCTAAGGCGAAGCCTAAGCGTAATCAATTAAAAAAAA	Maccastraga	SAMOROC	MODOTIC	CHOICE	CONTINU	COCCOCIC	STEMETER	PACCCCTAAGG	CACCCCCA	AAGGCGTAGTC	CATOCCANA	CACATTERATE		
Kpneumoniae 235.520		•												200
Minfluenzae 236.8EQ								£ 0	£	4				
Bhrochiseptica 238.SEQC			4	.aaa	Ö	Ü		0	£ 0	£ 5	E E	F		2 0
Bparapertussis 138.6EQ			F	8	Ü			Ö	E 0					700
g			6	8	Ü				4		5			
~	-			8	Ü	C. T.			4		C	Ų		200
Emallei 238.SEQ	.gc.	C.	H	8	Ü	CC.#	0	0	A			0		1397
Bpseudomallel 235.5EQ .GC				8	0	C.1.	0	•		:	:	Ö	Ü	1397
Monorrhoese 238.5EQ		. J C.	H 1	8		•	. C.T							1403
Membragastrones sassagement			A C T	•		Ü			A.		•	•••••••		1402
Paeruginosa 235.580	A.A.C.A.	.	:::::::::::::::::::::::::::::::::::::::	8		ACTGTT.	ę,		T		•	•••••••	• • • • • • • • • • • • • • • • • • • •	1392
Venotatae 438.520	.ATC.	•		:::::::::::::::::::::::::::::::::::::::	::::	•••••	• • • • • • • • • • • • • • • • • • • •	•			:::::::::::::::::::::::::::::::::::::::			1387
sencerocottetce 238.588.AA	Q.A A								F	•••••••				1404

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1410	420	1430	1440	145		1460	1470	1460
Bsubtille 235.SEQ	ACC. C. CACCATT - GA. A. A7	AT.		.AG.G.AAG.	0.0	TATT.		8. AGC	TA.00 1525
Banthracis 238.5EQ	ACC.C. TA. COTTTAINGA. :- TA TA	A T A	C	.A. GANGANG.		ATT.	0.AC.	K. MCG	TA.0C 1531
Efacaelle 238.8EQ	A.TT-, TOTITIANO-, ATA	ATA		A. GAATO	AT	ATF.	A. G. AT.	.cc.xocu.	GA.TC 1522
Llactis 238.SEG	A.A	TA		A. AGATG		AATTA	A.G.ATT.T.	J. CT. MCCA	
Lmonocytogenes 335.522ACA.TdTT-AMCTTTAG.G-AMTCAAM.GTGCCG.AGCACA.TG	2ACA.TOTTT-AAC.			.AG.G-AAT	5	.T. K			
Saureus 238.520	ACC.A.A-A.COTTTAATTCTA.G.CGAAGTA.G.CGAAGTATTA.TA.G.ACCT.ACCA.	T		A. G.COMO.		ATT.	A G. AC.	200	
	A.A	A		XC.A.MG	. L	XTF.	XX. XG. XC.	SCA. MCA.	
8	A.A	TA		xc.xvo	A.A.	XIT.	XX. XXT. T.	ca. 20Cs	GA.G. 1508
Spyogenes 138.580	A.A	TA.		XC.XX		ATT.	XX. MOT. C.	-oct.voch	
Mavium 235.SEQ	C.TOTA.GOGGGTCCTGATATCA.CTTAACCACCAA.ACCA.CGACCATT.CCCTTCGGTCGCGATTCGGGGGT.CGT.GGAC.TTC.C.GGTAGTAC.AA	ATCA. C T	EMOCROCCIA, ACC.	A. CONCOURT.	cerroad	. TOCCONTICOCCT.	SOT. OGAC. TTC.	C. COTACTA C	.A. A 1636
Ntwberculesis 218:520 C. Toro. GOGGGGGGGGGAA. C. T INACCACACA. A.C. A. CGA. CACT. CCT. CCT. TOGAGTTCTGGGGGCT. CGA. CTT. C. GGTAGTA. C. A. C.	C.TOTO.GOGGGCCCOTGA.	ATCA.CT	PACCACCCAA.ACC.	. A. CGA. CACT. C	xcrrcog1	. TOGNOTICTOGOCT.	ST. GGIA. TTC.	C. GGTAGTAC	.AQ 1656
Ecoli 218.SEQ	TOTOTINGS	GAAGGGGGGAC	GARGANG	OCTATOTTOOCC.	999	ZYC	OPTION COOR	TTTMOCOTOT.	AGGCT 1480
Kpneumoniae 238.SEQ				A				,	1478
Minfluenzae 235.530	AANG	T.T.		.TG AT.G	C		A.A.GTOC.	Tag	
Bbrochiseptica 235.8;	Bbrochleeptics 215.880ground.0ntrcrcc.Ga.G.Cur.Af.cff.cff.cff.c.f.c	T	JCCCTCCC	. CA. G CAT. A.			XCT.	ocrocar.	CANCA 1464
Bparapertussis 235.5	Bparapertussis 235.8EQ GTGGTNC.G	T	TCC	. CB. G. CAT. A.		. Transmannest.	XCT.	acrocar.	GWGN 1465
Spertussis 238.SEQ	GTCGTAC.0TTTCGC.GA.GCAT.AT.FTTALGTGAAGA.1465		2021	.CA.0CAT.A			MCT.	GCTOCAT.	GAGA 1465
Brepacia 235.880	ATTOT. AGAT	E	30003000	. CB. G		.1000.04.011TI1A-OI.,C.O.COCIOCAIG.AGA	XA-OTC.	o.cocrocar.	G. MGA 1469
Emalle	OTCOT. NANTT			. G. G		;;	X-OTC.	a.cocrocur.	G. AGA 1472
Bpseudomalled 235.5B	Bpseudamalle1 235.588		2001	G. G			X-OT. C.	G. COCTGCAT.	G. MON. 1472
Ngonorrhoese 238.5EQ	Ngonorrhoese 238.8EQA.TCA.A	2.7.		.T. G	D.A		MATAG. TT.	8	TG 1479
Neminigititels 238.8	Nominigititdis 238.882T.T			.TGT.	D.A	L'annanananan'i	MING.TT.	8	TO 1478
Paeruginosa 238.880	.CTG	TA.		0.0c		· 11/			1468
Vcholeras 238.820	.CIGACT	T		00	٠.٧			CT.C	1463
Yenterocolitica 218.5EQ	380 Gas			C.A					G 1480

FIG. 2B-14

1523

atossotis.c., 63., 63.a. cotiacuate, giget., ta...g...a. cootiag--agac..tag.ca.a.c.gic.etcactaatoctgargeataccott.a 1754 ANCOGOTO.C....GG...GGTA.CCGTACCAGTC.GTGGT..CA...G...AA.CCGGTAGGAAGC..TAG.CA.A.C.GTC.CTCACTAATCCTGAGAGGTGACCATAACCCGGTT.A 1776 Bbrochissptica 215.5EQT.0000TT.....-....................GXG----G.AT.AAG..T...-------GC-......CG.CC.AGTC-----------...T.GICTTITA.-----------.C.OG...OS.MT-MTA-----------...60..A.Q.A-.-TIM-----. TGAA. TG. . T. . - - . . . G. T~T. . T-----TCTAT---. . CAT CT. . . -------- GG. . . CG. AG--TTTA-----OSTITICCAOCCA--AATOCCCAAAA------ATC---AAGCCTGAGGCOTG-------ATGACGAGGCAGGAGCACTAG---G-----Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. ----ICICI----,.....A.AC.--------,C.T....CT.... T.A.AMOT......CTIA----TCG.G---...CT...---CTIAT------ICT.---...CA.C...AA...,------.C.....--TOT.TAC. 1530 T.AG.ATT......CT...--TCG.T----TCG.T----Lannocytogenes 238.5201.AdMA7....--....-CTTC-----TC.CG---...CA....CT...---TTGNG. AG. . TT.---. . . O. T-TT. C----TCT . T--- ACA . . TT . . . ---. Torgriot. T. . --. . . G. A-TITC-----TCT . T--- . . CAT. . . . CT . . . ---1510 T.000CTT.....--.....000C----1500 Tuesday, November 27, 2001 4:14 PM 1490 Neminigititels 238.529.MOACT Mcuberculosis 235.520 Bpseudomallei 238.SEQ Paeruginosa 238.8EQ Kpneumoniae 238.SEQ Hinfluenzae 238.880 Spneumoniae 235.520 Bpertuesis 238.820 Bsubtilis 218.5EQ Banthracis 218.5EQ Vcholerae 239.8BQ Efacaelis 23S.SEQ Spyogenes 218.880 Boopacia 218.820 Smutane 239.880 Llactis 238.52Q Saureus 239.52Q Emallei 235.520 Mavium 238.820 Ecoli 235.5EQ

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.

5	
ĩ	i
4	
Ċ	
4	1
5	į
ğ	ł
N	
Š	i
overnoer 27, 2001 4:14 PM	1
ğ	I
본	I
ĕ	ſ
8	l
Ž	ŧ
\$	I
Ē.	۱
Z	I
*	
×	I
JUBEDAY, NO	

1701	1708	1700	Year	1707	1707	1684	1688	1690		• •			1664	1630	1640	1640	1643	1646	1646	1658	1657	1648	
	Ų	Ü	U		Ċ		Ü	U	£	į.	TCACACAA	E								•	• • • • • • • • • • • • • • • • • • • •		
4	£	2 20	•	7	ON L	•	λ. Ε	7. F	î	ā	3300000		Ų					-	-	F	F		
O		T. C. 7	1 00		£	20 1	8	9.4		U	PAGNATAC			AT.	E EA	T TA	E	ţ-	+	£4	:		
5	5	5	5	A-TCA G	A AT	3	9.	8			T-CLOOTIN			C. O. AT	CA. O. AT	D AT	9Q.AT	P. Q. AT	G GA. AT	G-TAT	P	0	C
1	Y	¥.		, Y	4	4	4	Å			CLOSTOO.									•		:::::::::::::::::::::::::::::::::::::::	
E									ď		- 3								:::::::::::::::::::::::::::::::::::::::		: : : : : : : : : : : : : : : : : : : :	:::::	
O	0			U	O	0	0	Ö			COTACCC		- :		O	Ö	9	0	9		0		
G. COTOCC	A. OCTOCC	.A. 1000	A	STA. 1000	T. COTOCC	8.1.3	3C1.0	ACT.CC	\$6.88 \$30 \$30 \$30 \$30 \$30 \$30 \$30 \$30 \$30 \$30	ACA. GOCC	CATCHAI		J	7.8.6.0	7.0°.0°.F	2.0.0	ACCURG C	ACCUATO.C	ACCANTO.C	AG	BGR	9. 89.	A. CHCAGO
0	A	A	ZT	. MOT	3	T.A.T.	T.MC.T.	T.ATO.T	G C. F	0.00	TCACOTA	•	₹C	Ç	Ç	QC ·	121	ţ	152			:::::::::::::::::::::::::::::::::::::::	Z
8	8	98	8		S. C.				8	8	TCTANGCA	•	A		-	2	-			A	λ	E	
		:	:	•	•	E		:			CANADOC			:::::::::::::::::::::::::::::::::::::::	••••••	:::::::::::::::::::::::::::::::::::::::	•	:::::::::::::::::::::::::::::::::::::::				۲	
O	0	7. O. K	Z0X	5	8.0.1		J 0 J	7g	0		NOCTION		4									7	
7TN.	7	₩.T.X	₹2	3.17.₹	Ø. 11.×	7.T.D	73.C.T.X	727	.CC. 7.	10.C.T.	NATOCC:	4	Z . 4 . 9	TOUN-G	TOCK-O	TOUNO	TOGANC	TOCAL-O	TOUNO	4		•	Y
E	101	1		T	80. Joor	7.0	D. FT	P			avacuc			7.02	12.1	19.1		F		•		100	20
		ğ	8	SEGA.0	ម៉		•	ğ	Ŕ	SEQ .00	_		:	. SB000C	. SEGOOC		¥	ğ	000 000 000 000 000 000 000 000 000 00	ខ្ល	580 CK	o o	
138.880	238.820	38.8EQ	3.880	knes 238	3.880	3.580	3 23S.SE	33.50	28	11s 23S.	SI,	1 238.SE	1 23S.8E	tice 238	1818 23S	238.820	38.820	S. 820	let 238.	ae 238.S	Edis 238	A 238.88	238.820
be1118 2	thracis	caelis :	otis 238	nocytoge	reus 235	tens 238	enmonta	. seuedo	16 23S.	Derculo	11 238.1	• mounter	Lluenza	ochisepi	rapertui	rtussis	Pacia 2:	1104 231	eudoma1.	norrhoe	inigita	Enginos	Vcholerse 238.520
	ACC 1715. 170 T ACA G A	AGG TTC. TG. T ACA G A	ACC. TTC.TG.T. A.CA. G. A	ACC. TTC. TG. T. ACA. G. A	ACC. TTC. TG. T. ACA. G. A	ACC. TTC. TG. T. ACA. G. A	ACC. TTC.TG.T. ACA. G. A GOD G. GOTGCC G A. G A. G T. A. C A. A. G G A. A. G G A. A. G A. A. G G A. A	ACC. TTC.TG. T. ACA. G. A. GOD G. G. GOTGCC	ACC. TTC.TG. T. ACA. G. A. A. GOD G. G. GOTGCC. G. T. A. G GA. G. C. T. T. A. C. ACC. TTC.TG. T. ACA. G. A. T. GGG A. A. GOTGCCT. G. T. A. G GA. G. T. C. T. T. ACC. T. ACC. T. ACT. G. A. T. A. T. A. T. A. T. T. A. T. T. A. C. T. C. T. ACC. T. AC	ACC. TTC, TG T. ACA. G. A. GGG A. G. GOTGCC. G. G. GOTGCC. G. G	ACC. TTC, TG T. ACA. G. A. GGG A. GGTGCC. G. G. GA. G.	ACC. TTC, TG. T. A.C. G. G. T. A.C. G. T. T. A.C. ACC. TTC, TG. T. GGG A. A. GTGCCT. G. T. C. T. T. TGC C. T. T. TGC C. T. T. TGC C. T. T. TGC C. T. T. A.C. C. T. T. T. A.C. C. T. T. A.C. C. T. T. T. A.C. C. T. T. A.C. C. T. T. A.C. C. T. T.	ACC. TTC.TG. T. ACA. G. A. TCC. CTC. T. ACA. G. A. ACC. TTC.TG. TC. T. ACA. G.	ACC. TTC.TG. T. ACA. G. A. TCC. CTC. T. ACA. G. A. TCC. CTC. T. ACC. G. TCC. CTC. T. ACC. G. A. TCC. CTC. T. ACC. G. A. TCC. CTC. T. ACC. G. A. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. G. A. ACC. TTC.TG. T. ACC. G. A. ACC. TTC.TG. T. ACC. G. A. ACC. TTC.TG. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC. T. ACC. T. ACC. T. ACC. T. C. ACC. TTC.TG. T. ACC.	MCC	MCC TTC, TG, TC, ACA, G, A, A, GOTGCCC G, A, A, GOTGCC G, A, A, GOTGC G, G, A, GOTGC G, G, A, GOTGC G, G, A, G,	MCC TTC, TG, TC, TG, TC, TG, TC, TG, TC, TG, TC, TG, TG, TG, TG, TG, TG, TG, TG, TG, TG	MCC TTC. TO T. A.C. G. A. T. GCO A. G. GOTTOCC. G. T. A. G. G. G. G. G. T. C. T. T. A. C. G. A. G. G. G. G. G. G. T. C. T. A. G. G. G. G. G. G. G. T. C. T. A. G. T. G. T. A. G.	MGC TTC, TD, .T. , MCA, .G	MCC TTC, TTC, TT, LT, LCA, .GA. GCG	MGC TTC, TG T, ACA G A A G G G G G G	AGC STEL TO AGC A A GOTOS A A A A A A A A A	AGC TTC, TG, TC, ACA, G, A, A, GGGGGG, G, T, A, GG, GG, G, G, T, T, AC, G, G, G, G, T, T, AC, G, A, A, G, GG, G, G, T, T, AC, G, A, A, G, GG, G, G, T, T, AC, G, A, A, G, GG, G, G, T, T, AC, G, A, A, G, GG, T, G, T, T, AC, G, A, A, G, GG, T, G, T, T, AC, G, A, A, G, GG, T, G, T, AC, G, A, A, G, GG, T, G, T, AC, G, T, AC, G, A, A, G, GG, T, G, T, AC, G, G, T, AC, G,

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1
	-
Σ	į
7 ∑	١
4	ı
4:14	ı
4	Į
\leq	1
5	ł
N	ı
٧,	ı
27, 2001	ı
≂	ł
	İ
듗	Į
>	Į
9	Ì
_	ı
≥	ı
8088	
2	ı
š	ı
_	1

	1660	1620	1680	169	- 5	- 5	- 5	9555	- 5		- 5	 	
					3	2		4.30	7.40	00/1	00/1	2/1	
Baubtilis 235.SEQ	7CT	 U				oorG	crororoc		8	000 C	TO.AT	A CCG, GAG., C., C., TG, AT., GCCCAG., G	1805
Banthracis 235.500	4t		300	• • • • • • • • • • • • • • • • • • • •	•	.oct	C	****	YC.	GNACCTG.ATOCCCMG.	TG.AT.	CCCM. G	1801
Efacaelis 238.8EQ	£5	υ:		• • • • • • • • • • • • • • • • • • • •		į		į	60.70		TG.AT.	SCOOK G	1791
Llactis 238.520	763	S		• • • • • • • • • • • • • • • • • • • •	•	.aor	TOT		V A		.TO.AT.	3000	1779
Imonocytogenes 238.SEQTCT	orcr	Ü	X	• • • • • • • • • • • • • • • • • • • •	:		CTATOTOC-	1	88		.TG.ATQ	300000	1811
Saureus 215.SEQ	7CTC.	C	χχ			.cor	CT-TGTT	į	-3CCG.	ACCC.0.MGCCTG.ATGCCCAAG	TO.AT.	SCCOM. G.	1805
Smutans 235.520	£		8	•	:	2	06G.CGAT.	***************************************		A-, TC, C., C., TG, AA., GCCCAA	.TG.AAG	XXXX	1776
Spneumoniae 238.SEQ		 	78	•••••••	:		GAC. T	***************************************	-ATC	A-, TCCCTG.ATGCCCAA.	.TG.ATG	XXXXX	1780
Spyogenes 238.580	£			• • • • • • • • • • • • • • • • • • • •			.OGTGAC. T. G	1	54	, TC, C., C., TG. AT., OCCCAA.	.TG. MTG	XXXX	1781
Mavium 238.550			8	• • • • • • • • • • • • • • • • • • • •		20C.C.C	.xcc	cocc.c.chhccchTcTgg.g.rg.cchTgThc	0.0	7O.CC.	Α	T.A G	1990
Mtuberculosis 219.580 TT	4		88	•	:	POCA. C. CA.	.A.C	606A.C.GAA.CCATT6d.G.TC.GCATGA.GAG.	0.0	TC.QC.		10. csc.	
Ecoli 218.880	CONTEMBERACT	WCTXXX	SALATOGRADO	STANCTTCOOGA	A	CACOCTOAT	TOTAGGTCA	SAGGNAINGTOCCOTAACTTCCGAAAAGGCACGCTGATATGTAGTGAAGTCCCTCGCGATGAACTGAAATGAGGGAAAAAAAA	GCACCTGA	ATCAGTOGAN	GATACCAC	TOCTOCKACT	CTT 1777
Kpneumoniae 235.5EQ	• • • • • • • • • • • • • • • • • • • •		• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	:	9			•	0'0	•		1775
Hinfluenzas 236.620	,		5 K		:	76c.90	J.A-CAT. T.	TGC.GCG.A-GATT.AGGGACCCAG.TGC.G	A. 0. TO	.0.0	:		:
Shrochiseptics 238.850AA	Q.YA.		TA.A.	• • • • • • • • • • • • • • • • • • • •	`:	7.TA.CC	OTord.	.T.TA.CCTGTGTGAG.CTCGCTATGGGGCG.ATC.G	TATG	.000	0.ATC.0	3g	
Bparapertussis 238.550AA	0AA.		TA.A.	• • • • • • • • • • • • • • • • • • • •		T. TA. C - CTO	O TOTO.	.TOTG MG. CT CGCT ATG GOG C G. ATC. G.	DIA	000	O.ATC.	9	1756
Spertussis 238.520	λλ	ີ່	TA.A.	•••••••	•	1.TA.CC	10 TOTO	T.TA.CCTGTGTGAG.CTCGCTAIGGGGCG.AIC.G.	TATO	.000c.	O.ARC.O.	:	1756
Beepacia 238.5EQ	.	O			F	.T	rc.13	.C. TG.C.00.CTCCA00TG000T.C.ATA.A.T.G.	0000	.000. T.C.	ATA.A.T.C	3	1761
Brallei 218.85Q			T		H	T	:	.c.15.c.00.c10c1.1.	•	GOTG GGG T.C.ATA.A.T.G.	ATA.A.T.O	0	1764
Bpseudomallel 218.SEQA		Ü			£.	7r	gc.12	.fCCTGC.TG.C.GO.CTCCA.AGOTGOGGT.C.ATA.A.T.G.	1.A COTO	.086. 1.0.	ATA.A.T.C	30	1764
Ngonorrhoese 238.550 A	٠٠٠٠		TA.A	• • • • • • • • • • • • • • • • • • • •	:	T.T. C CTCTA.	_	TGATTCCFTACCCGGAGGCG.ATG	8	ZONOGC.	G. AT C	6.	2775
Neminigititdim 238.SEQAA	DAA.	Ü	TA.A		:	T.T. C-C	C-CTCTA T.	.TGATTCCOTACCCGGAGGCG.ATG.	₩₩	Agradac.	0.XTC	9	1774
Paeruginosa 235.5EQ			5		:	TG C. GCIMO.	CINO.	GATT. ACTOOD. A		croocro	38		1765
Vcholerae 238.550						TCTAT	AT				,		1760
Yenterocolitica 218.5Eq			•		:				:				1780

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Bautitils 218.582		1780	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	
Banthacis 135.5EQ GC AT	Beubrills 238.580	8	Q.	0.87	8	8.0		6		. G. C. T.	.F.	G. 6250. F.	<u>.</u> 	921
Efecatifs 218.520	Danthracis 239.520	8		00				E		NGA T	<u>.</u>	6		1017
Limetis 218.5EQ	Efacaelis 219.5EQ			ATT	ATA	8		£	3		7700	r vol		1907
Lunch cycogous 215.5EQ	Llactis 238.SEQ	:::	a	TT		0		£	3	1.GA.T.C.TXC	. T T-C.	£ 0		1897
Seureus 23S.SEQ Seureus 23S.SEQ Seureus 23S.SEQ C. G. M. T. AT. A. G. G. Senutans 23S.SEQ C. G. M. T. AT. A. T. G. G. Senutans 23S.SEQ C. G. M. T. AT. T. G. G. Spyopensons 23S.SEQ C. G. M. C. T. AT. T. T. G. G. Revium 23S.SEQ C. G. Revium 23S.SEQ Spyopensons 23S.SEQ C. G. Revium 23S.SEQ Sprottus 23S.SEQ Sprottus 23S.SEQ Sprottus 23S.SEQ Sprottus 23S.SEQ Sprottus 23S.SEQ Special	Lmonocytogenes 235.SEQ	8	g	XCT	5	0	• • • • • • • • • • • • • • • • • • • •	E	3	1.Q.T.C.T	-1100C.	G. ACCA. 7.		1927
Smutans 235.5EQ C.C.T.AC.TT.AT.A.T.A.G.G.G.T.T.C.T.AC.TT.AC.	Saureus 235.SEQ		g	T MC T	T	86		H		J. GA. T T A	-TTCTC.	S		1921
Spreameniae 215.5EQC. T. AT. T. AT. TGGGA. CA. TCTCTCTCTCT	Smutans 238.520		-	GATT	AT A.	0		E	9	J. GA. C. C. T MC	TTT. T.C.			1894
Spycgenes 235.5EQ . C. T. AT. T. AT. T. Q. Q. G. T. C. T. AT. T. AT. T. AC. AC. AC. AC. AC. AC. AC. AC. AC. AC	Spneumoniae 235.SEQ		•		AT T.	0		£+	3	J. CA. T. C. T		40.40		1896
Mavium 215.8EQ .CCA. GT. C. AC. T. AC. .CCA. GACCC T.A.CC T.A.CC .C. AC. .C.	Spyogenes 235.5EQ		-	TT	AT T.	0.0		E	9	. G 7.7 A	Ų.	3		1897
Mtuberculosis 235.5EQ	Mavium 235.SEQ		g	D 19 0		•		H	:	A. GACCC T.A. C	C. 7.	T ADADO		
Ecoli 235.5Eq	. Mtuberculosis 238,5EQ		g	G9		2	:	E	3	LGACCC. T.A.C	8	1000		132
Kunfluense 238.8EQ C G T T.T.C-T.A. Minfluense 238.8EQ C G C G T T T.T.C-T.A. A T	Ecoli 238.8EQ	TATTABASA	cicacacata	CAMCACA	NOTOGRACO	'ATACGGTGT	MCCCTCCC	GTCCCC	TAXTTOON	TGATCCCOTCACC-	-octageeast	OCTOTATA TO		1803
C C C C C C C C C C C C C C C C C C C	Kpneumoniae 238.SEQ	:	•	•							4-0-F			0 0
C C C C C C C C C C C C C C C C C C C	Hinfluenzae 238.520	•	•	:		b	E	E		7	A-04	0		0 0
C C C C C C C C C C C C C C C C C C C	Bbrochiseptica 238.8EQ	•	:		C	0			U					6 6 9 8
C C C C C C C C C C C C C C C C C C C	Bparapertuseis 238.880	• • • • • • • • • • • • • • • • • • • •	:	9	2	0			O					
C C C C C C C C C C C C C C C C C C C	Bpertussis 235.5EQ	•	:			9			O					3 5
C T T C AT	Bospacia 238.SEQ		:			9			A A			H		1868
6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Bmallel 235.520		:			0			AA				£-	1871
	Spacudomailet 235.550	.	:					:::::::::::::::::::::::::::::::::::::::	٨٠٠٠٧			4	T 16	1871
	Mgonorrhoese 235.8EQ		:						: : : : : : : : : : : : : : : : : : : :	A.AI		ATCG		1882
2. SEC	Nemangicicots 235.880	:	:		•	9		•	:::::::::::::::::::::::::::::::::::::::	A.AT	AG	ATCG	77	1881
	Faeruganosa 238.5EQ	: : : : : : :							:	Υ		•	T	1881
Verterocolitics 238 SEC	Venoterte 235.820	•		AT	X T					J				1876
	Yenterocolitica 238.5E			•				-		•			T	1896

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1800	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
Baubtilia 215.530	.A.						Ü	4	00	1886		-
Benthracks 238.550	.A	:		• • • • • • • • • • • • • • • • • • • •		: : : : : : : : : : : : : : : : : : : :	υ		CT	TOOCH	ATCTJG3GCAAA.	O
56566118, 238, 52Q	Α	•	•	••••••				A	C7		3	U
244CL18 438.3EQ	· · · · · · · · · · · · · · · · · · ·						0	K	-	TOCOCIA	A Ch.	c
Lmonocytogenes 238.SEQ.A		••••••				•	Ü	Α	Ü		4	
Saureus 238,8EQ	.A	••••••	• • • • • • • • • • • • • • • • • • • •				C	d				:
Smutens 235.550	.A	:						: : :	•			
Spneumentae 238.SEQ	A.	:						:		TARKE.		5
Spyogenes 215.520						· · · · · · · · · · · · · · · · · · ·	; ·		4	:		
Mavium 23S.SBO	2	:_	:		•	:		· · · · · · · · · · · ·	T	100CF	. TTGGCAA. GAG.	
uberculosts 238 SEO	4	2	: 5 6	•	:	:	•		ti	3		0.0
BASIL 325 SEE			5	• • • • • • • • • • • • • • • • • • • •		:::::::	•		E	T.TC&		0.0.
		STATE OF THE STATE	ATMOSCACCT.	MOOTHOCCA	LATICOTION	COCCINICI	COCHOCIAC	NOGMATOOCC	TAATGATG	OCCADOCTOTO	OUZIAMEGOGIACOTIANGOGICCIAAGGIACGIAATICCITGICGGAAATICCIGCIGCACCIGCACGAATGGGGTAATGAGGCAAGGAGAAGAAAGAAAAAAAA	C COMPANY
Kpneumoniae 218.520			• • • • • • • • • • • • • • • • • • • •									
Hinfluenzae 238.980	.А	_						•	•	• • • • • • • • • • • • • • • • • • • •		
Bbrochiseptica 238,880								•	••••			::::::
Boarapartussis 218 ggo				•	•		•		:::	5		
Branting of 136 and						**********	:::::::::::::::::::::::::::::::::::::::		:	5		
Care 435.640	•					• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	••••••		ថ		U
BCepacia 238.820	••••••		• • • • • • • • • • • • • • • • • • • •	•••••••		•		້ ປ	2	ð		U
DESTRUCTIONS			••••••			•	:	•	ฮ์	ฮ	•	C
Bpseudomellet 238.820			• • • • • • • • • • • • • • • • • • • •	••••••				0	0	đ	•	ı
Ngonorthoese 238.880							·					;
Neminigititais 238.880.A.	Α.									5	٠	
Paemidinese 218 SEC					•		;	***************************************		T	-•	
100 Oct 100 Oct				••••••	: : : : : : : : : : : : : : : : : : : :	:::::::::::::::::::::::::::::::::::::::	:				•••••••	
ACTION REFER 738.880			• • • • • • • • • • • • • • • • • • • •	•								

Alignment Report of Gram +&- 23S align.MEG, using Ciustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	2020	2030	2040		2050	2060	2070	3080	2090	2200	2110	2120	2130	
Baubtilis 238.880	AT. G. AC		5	Ø .4	:		1:	g	1.2	T.T. TG. OTA MCT. MC	Į Į		6	23.63
Banthracia 238.520	AT. G. AC.		5	AG.	:		:	0	C. T.TT	TT. GTA. AGTT.	ort.		0	2157
Llactis 235.580	77 G AC		5 8	0 Q			A0.0.	0	7.7.		χ χ		 .	2147
Lmonocytogenes 238.5EQAr.G.AC	AT.O.AC.		δ	. ✓			0	• .		GC. B.C. T.T. T. ST. CCTURAG.CAC.	9 9 0 0	:		2137
Saureus 238.880	CAT.0.3C	•	5	:	0		0		E	GCT.TAT.C.GCA. BOCT	2	*		7167
Smutane 238,820	71.0.XC		8		•		6.6	•	4.4	.GCTT.TCGTCTGTTACAC.	Ş	: :		2134
Spyconnia 218, 920	74.0.7C		£ 8			~~~~	ÀG.G	•	T.T.	GCTT.TOTG.CTGTA.CAC.			TX.	2136
. Mavium 235.880	AGA.		100 M		: -		A. 0.0	8	3.4.4.0	OC. TT.T GT. CTOTA, CAC.	, y	A.		2137
Mtuberculosis 238.520CAccaATcor	SZ.	, Y	þ	0				4 4	5				.	2349
Ecoli 238.880	ATTGAACTOGC	TOTOMORT	CASTOTA	2000000	MEMOCER	AMGACCCC	TGAACCTT	ACTATACCT	GICACTICAL	CATTGACCE	TCATOTOT	ATTGAACTCGCTGTGAAGATGCAGTGACCGCGCGAAGAGAGGCCCGTGAACCTTTACTATAGCTTGACACCTTGAAGCTTGAAGCTTGAAGCTTGAAGATGAAAAAAAA		4274
Kpneumoniae 23S.SEQ		:::::::::::::::::::::::::::::::::::::::			:	:								2133
Districtions 6 0 238.580		:		*******		•		•			•		U	2139
Boarabertussis 238, SBOG G. Cyr.				:				:	10. T. G.	.TGTGTGA GGCC.	98			2102
Bpertuesis 235.580	00	F	, C			4	0	:	16.11.0. 10.11.0.	TO. T. G TO A COCC.	9	• • • • • • • • • • • • • • • • • • • •	8	2103
Bospacia 238.880	G0.0TT	F	A.C.	F	5.	4	4					•	ģ	2103
Bmallei 238.520 GG.Off	00.ott	E	A.C.	_		Α	0		10. T	T A. GATC.	,		* *	2111
Monorthogae 218,880 GG.GIT		H	.TA.C	E 1		Ž		:	16TG.	TG. TGT A GATC.	MTC	•	A	2111
Neminigititeds 238.82000.0.T.	0.0.0							9 0	5. H. H. B.	10. 1. 6. 1 AGICACT.	Ę	•	6.	2122
Pasruginoss 238.820	A			F-				9 0	20.1		į		¥	2121
Vcholerae 235.880	····· A ·····	•	•	£					Ö	Ž.	9		4	2116
renterocolitics 238.550	ρ.				:::::::::::::::::::::::::::::::::::::::			•					. A. A.	2136

Alignment Report of Gram +&- 23S align.MEG, using Ciustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

•	2140	2150	21,60	21,70	2180	21,90	2200	2210	2220	2230	2240	2250
Baubtilis 218.520	skxk.	CT00T.	G.AT.03.	.CTQTG.AT.QGQGTQQCTQTACC.	OCCIOIA.	:	coccoc.1acoc.ax.	C.TG	90.000 1000	Α	O.	2278
Benthracis 218.520	•		.cr007acrasos		GGACTOTAAA	**	.c. A03001	0.10	C. Accort. T GACCCS. GA	λ	C	2374
Efacaelis 238.8EQ G			T00AGCT000GT		GTO. TA. GA. CCC.	:		C.A0		A.AC		3264
Llactis 238.5EQ			TTATTQTTQOG.	£	6.C.TA.G.T.AC.	3.T.MC	.cc.cago.	A.A O	.cc.cT00.A.A 0.0CMG. GA		AC.	2254
338.8EQ		.CL103A	ChACSA 0.A. TCO 00T		OCCIGIA. GACCA.	MCCA	8.8	C. T G.0	.cc.cca.gc.tg.gc.tog.ga	AC.		2284
Saureus 235.550	.ACCTAG T.	.crtor	crcor		. MCTOTG OCT.			C.T0	.cc.cAc.rorg.gA	ACC		2278
		TMC.TGTTG	G 1710		OTC A. G. C. AC.	J.C.AC		7A	.cctagotatctac.ga		<u>.</u>	2251
ន			T GWAA. CTGGGT		GTG. TA. G. CCAC.	3.00cc			.ccatagcta.c.ga			2253
Spyogenes 238.520	Crac	.T CANT.	0	.T GAATGTTGGOTGTG.TA.G.C.AC	OTG. TA.	3.C.AC	. S		.CCATMG . T CTA. C. GA			2254
		TTG-TTTTG		£	TGATCOTAGACAC	.cocsc	CAC.TGT.CA	0.110	5.5			2466
Mtuberculosis 238.5EQ .ACCC		10.00-CTT16.			CATCOTA.		.C.CA.	C A O		CC	4	2489
	AD-TOTOGACOCCA	orcrocar-o	MOCCEANCETT	MATACCACC	TITAMENT	GATGITCIA	ACCITICANCE	XXXX-TCC	PLOTOTOCKT-GONGCTTGAAATACCACCTTTAATOTTTGATTCTAACGTGGACCCGTGA-TCCGGGTTGCGGACATGTGTGGTGGTAGGTAGTTTGACTG	Agranciagia	SOTHOTTTONC	700 2250
	Ÿ	0				••••••	7.0	0.0.0.0	1.66.66.6		••••••	2250
Hinfluenzae 215.520	-Coo	ATTG		AITG.*G			ATO	CCAA.O		••••••	••••••	2256
Bbrochiseptica 238.880.CTCA.T		.Ar040.	At CMG AT	:			C.T.OTTA.C.G	7.	A.C.G	Q		2219
Bparapertuseis 235.520.CTCA.T	_	AT GMG	AT	• • • • • • • • • • • • • • • • • • • •	Serie.		.C.T.O.		.C.T.GTTA.C.G	CAAC		3220
8	.crcA. T	AS CMG.	AT	• • • • • • • • • • • • • • • • • • • •		:	. C. T. 93.	4.	C.T.OTTA.C.GCAA.C	ชฮ		2220
_	.ACCATTGOT		H			0	C.T.G			QAC.	c	2228
Bmalle1 238.520	.ACC A.TTT QQT	.T00T.					C.T.G		6.9	Q		2228
Bpseudomalle1 238.82Q .ACcAT.	.XCAT.	T 00T	£-		oort	:	C.T.O					2228
Ngonorrhoese 238.5EQCAA		TorT				•	8	C	CCA	ري دي		2239
Neminigititéis 235.550CAA		101	H		oora.c		8		CC.GCAAC	4Q		2238
Paeruginose 238.520		100	X f		.00CCG		.177.04.	Α	TCTOTAA.C.AA	A		2238
· Vcholerae 235.520	. AC	.TG.T.			5	•	T. A.		Α	c.	• • • • • • • • • • • • • • • • • • • •	2233
Yenterocolitica 238.820			A			•	g	A	TCCA.TGAA	A		2253

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	2260	2270	0 2280	2290	9	2300	9.65	2320	2330		2340	2350	2360	25.50	
Baubtilis 218.522	g	R			8.	.ccc. a.A T A		TTC.CMGTAC06	4.7	0			ACCT. MOT.	1	2398
~	g	-	CCA	A A	8	CC. C. G.A T A		TTC. 230	Υ	δ.		X	ACCT MAGE.		2394
Efacaelis 238.5EQ	0	. X	:	Α	.cc85	GAST	T			0		X	ACCT NACT		2384
Llactis 238.5EQ		:		A	C. CAT.	:	-	ATC. TAG T A T. A.	T.	τ.λ.		4	A CT. 23. T.		2373
Lmonocytogenes 238.SEQG	6	:		A	8			TC.CG.	ď.	.T. A C 00		Q	AC MOT		2404
Saureus 238, SEQ		2	K.T O	A	.cccc.	GAAT	T A	TTCATAGTA	T. A	ð		X	ACCT. MOT.		2398
Smutens 235.5EQ			:		8	.cc.chtA.	Υ	.TC.TMG.	T. A.	T. A. T.		24	ACA MAGT.		2371
Spneumoniae 218.8EQ		2		λ	8.	.cc.a	4	.TC.C.6.	T. A.	T. A. T	:		A.CT. 28.7		2373
Spyogenes 235.550		:		A	8.	GATT		.CCC.GATTAATC.CAGTATGG.	T.A.	9			A.CTM.T.		2374
Mavium 238.520		AT		Α	8.	AA.	ð		5	Ü		X	AC.T. ANGT. A.		2586
23S.8EQ	gg.	7		Α	8.0	AA.	ð	8	T. AT.	0		X	T. ANGT.		2609
	COCCUCTOCTION		манстилосански самасттостилестватест статесанскате на предержина се предержина предвижение в предвижение в пре	CONCI	TOCCTAN	recreate	COLCATO	CONCOLLING	TOCAATO	CENCO	SCTICA	CTOCCMOCC	TOVOSCOCO	6	2370
			• • • • • • • • • • • • • • • • • • • •		A	:				•					0212
Hinfluensae 235.820			••••••		-	XC		5		F	4		ACA. ANOT		2376
Bbrochiseptica 238.520	9		#			•	A	A91.CT.A			£	2	7 - 7 - 7 - 7 - 7 - 7 - 7 - 7 - 7 - 7 -	: :	2337
Sparapertussis 338.SEQ	J	Ü	4		AC GOTAC.	•	٠٠٠٨٠٠				K	7	7 P. T. A. D.	4	2339
Bpertussis 238.820			:	:	AC GOTAC.	:	A	₹.£.			£	7	F- Y 24	4	2110
Bospacia 238.520		•	-			COTAC	₹	₹. £3. £5.			8	ş	C. MGT		2345
Bmallet 238.820	9	0		:	XC	cornc	A	. A. C. C. A.			¥				2348
Bpseudomalle1 235.SEQ	J	0			g	COTAC	A		•		¥.	:	•		2348
Ngonorrhoese 238.520	9	0		.T	3		AG.ACT.A.	3.ACT.A		4	4	8	•		2359
Neminigititals 238.880	·····		£				A	A O. ACT. A	•	\$	F A	8			2358
Paeruginosa 238,820					T	•	A	G. TC. CAG AT A A GC.	. AT A.	Α	×				2358
Vcholerae 238.520			• • • • • • • • • • • • • • • • • • • •	:	0	XC1	8	£			C				2353
Venterocolitica 238.520	y			:	A	X C	•	:	, , , , , , , , , , , , , , , , , , ,						2373

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

-		2380	2390	2400	2410.	2420	2430	2440	2450	3460	2470	2480	
Baubtilie 235.8EQ	3	QATCG.T						00			ľ	C. C	2518
Benthracie 238.920	.a	TCO. T.			•	•••••••	• • • • • • • • • • • • • • • • • • • •					SK.C.	2513
Efacaelis 235.SEQ	GATCO.T	70.07	•	0.0	c.c	55		.cc.T				. O. O	2502
Llactis 238.550		T T.	:		:::::::::::::::::::::::::::::::::::::::	• • • • • • • • • • • • • • • • • • • •		.CC. T				. C	
sonocytogenes 238.	BOCK	TC0.T.				• • • • • • • • • • • • • • • • • • • •			• • • • • • • • • • • • • • • • • • • •	·····		C. C	
iureus 238.880	5	AC. A.T.			:	•••••••	• • • • • • • • • • • • • • • • • • • •			0.10 E			
Smutans 335.520 GATCO.T	3	TC0. T.	:	A.C.T.		T.O O			••••••			.TCT. C	
Spneumoniae 238.SEQ	ฮ	G		C.#.	:			C C. T.	• • • • • • • • • • • • • • • • • • • •	TCT.C		C	
spyogenes 238.5mg	.	70.7	:		:		: : : : : : : : : : : : : : : : : : : :	.CC.T.		CC.TTCT.C	•		2493
MAVIUM 438.58	5		:			OTO .A.	• • • • • • • • • • • • • • • • • • • •						2705
membereulosis 438.580 GATCCAT.	5	TC. GAT.		CACCC.CG		919	• • • • • • • • • • • • • • • • • • • •					C	2728
ECO11 435.5M	TOCOMO	TOGANICO CONTRA	Madurca	STOSTICICAL	ATOCIMOO	SCARCETCA	ACCCATALAN	COTACTOCO	SCENTANCIO	CTGATACCGCC	PAGAG-1	KATAKTOTOTOTOTICHATICHANGCARGCCATGGTTARGATAGTAGTACTCCGGGGGTTARCAGGCTGATACGGCCCAAGAG-TTCATATCGACGGGG	3 2489
Aprilemental 135.822					:::::::::::::::::::::::::::::::::::::::	:		• • • • • • • • • • • • • • • • • • • •			:		2489
niniivensee 638.660			•		:::::::::::::::::::::::::::::::::::::::		•	•	•		•	***************************************	2495
Described to the second of the					:::::::::::::::::::::::::::::::::::::::			•	·····		,		2451
bparapartuments 432.320				,							•	***************************************	2453
Speciments 438.000		·····	:::::::::::::::::::::::::::::::::::::::			••••••••		-			•		2453
Bunllet 238.5E0			:		:	•••••••	• • • • • • • • • • • • • • • • • • • •						. 2464
Nonewidowalled 214 SWO	ç					• • • • • • • • • • • • • • • • • • • •		# 1	•				. 2467
		•••••		H	:	• • • • • • • • • • • • • • • • • • • •					· · · · · · · · · · · · · · · · · · ·	•••••••••••••••••	. 2467
Ngonorrandes 436.654					:::::			•	• • • • • • • • • • • • • • • • • • • •				. 2478
Nominagicated 438.850		· · · ·		£0.1	:			••••••				* ······	. 3477
Teruginose 433.884	-	.A T T.	•	T	:::::::::::::::::::::::::::::::::::::::	•••••••••••••••		• • • • • • • • • • • • • • • • • • • •			•	•••••••••••••••••••••••••••••••••••••••	. 2477
Venoteree 435.88Q				F	: : : : : : : : : : : : : : : : : : : :			•		•			. 2472
IONICETOCOLICIOS AUSTROAMONTTT.	DECA.	T T.	******										

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

26	2490 2500	2510	2520	2530	2540	2550	2560	2570	2580	. 80	2600	
Baubtilia 235.550		ο		TC.		5	ð					2637
Banthracis 238.520	•••••••				:::::::::::::::::::::::::::::::::::::::		:	, i				2632
	•••••••	0			•	:	:	0.0.				2621
Llactis 238.8EQ	• • • • • • • • • • • • • • • • • • • •	90	:	TC			Υ	00.				2610
Lmonocytogenes 238.820	•••••••	0		TC	2	2	5					2642
S4ureus 235.8EQ		.0		JF		•		.c.				2636
Smutens 235.8EQ			:	•		p	ð	.CC				2609
Spneumoniae 238.520	• • • • • • • • • • • • • • • • • • • •	:	:	•		ರರ	ฮ์					2611
Spyrogemes 218.520	••••••••		•		g	g	•		g			2612
Mavies 238.880	• • • • • • • • • • • • • • • • • • • •	GG.	:		A	:	ฮ์					2824
235.880				60		g	đ		• • • • • • • • • • • • • • • • • • • •		•	. 3847
Bcolf 215.5EQ	TTTOOCHOCTOOKE	IOTOCOCTCATCA	CATCCTODOO	CTONOTAGOT	COCANGOG	PATGOCHGTTO	DOCUTTAN	CONTROP	WASTEROOFFERM	A-concor	OTCOCTICATICATICATOCAGO OCTANOTAGOTOCCAAGO GAATATGC TACACAATTAAAGA GAAGACAGO GAATTAAAA -COTCOTOAGAACAGATTOG GA	•••
Kpneumoniae 218.880	•••••					•		•	•			2608
Hinfluentee 238.820											• • • • • • • • • • • • • • • • • • • •	. 2614
Shrochiseptics 238.88g				•				. A		A.	F	. 2569
Bparapertussis 235.552	• • • • • • • • • • • • • • • • • • • •		:	35	::::			.A		A.		. 2571
Bpertuesis 235.SEQ			:	3				£	:::::::::::::::::::::::::::::::::::::::	A		. 2570
Bompacia 238.5EQ				8		•	: : : : : : : : : : : : : : : : : : : :	F Y	~	٠		
Brallei 238.8EQ		:::::::::::::::::::::::::::::::::::::::		48					·			. 2586
Spseudomellei 235.5EQ			:::::::::::::::::::::::::::::::::::::::	48		•		F 4.	.T			. 2586
Ngonorrhoese 218.5EQ								£	A			. 2597
Neminigititdie 238.5EQ								4	,	;;;		. 2596
Paeruginosa 218.5EQ				8				• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •			. 2596
Vcholerae 235.5EQ				Ü			:		•••••••		•••••••	. 2591
Yenterocolitica 235.SEQ	g								•		• • • • • • • • • • • • • • • • • • • •	. 2611
		•										

FIG. 2B-24

Alignment Report of Gram +&• 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

	- 55	2636	- 52	,	250	- 55	1000	- 30	9856	<u> </u>	ŀ		
•	2	4040	0507	? _	007	20-	0/97) 0 7	0607	2 •	07/7	07/7	9
Baubtilis 235.8EQ)	C.T.C.	A. TT.	AA	10.T		3	0.0	5	ğ	63	5 J	70 2755
Banthracis 238.820	C. 7	:	TA A. TT.	AA	.TC.T			:	:	:	0	TA. TG.	
Efacaelis 238.520		:		AATC.T.	70.7	•	:	:	:		9	2	
Liactia 238.8EQ		:	.TATA. TT	AAT	£.5.		g	:	:	32	3	ز	
Imonocytogenes 235.8EQC.TC.		C.TC	λλ.π.	AA	.TC.T.		:	:	:	:	500	77.75	TO 2760
Saureus 238.5EQ	C. H.	:	TA A. TT	AATC.T.	7.7.		:	:	٠٠٠ ١٥٠ ١٥٠	:	•	.T.ATG.	70 2754
Smutens 238.SEQ	C.T.,C.	:		AAT		:::::::::::::::::::::::::::::::::::::::		:	:	٠	£ 3		7272
Spneumoniae 138.SEQ	•	C.3C	.T.A A. TT	AAT	••••••	:	Α	:	:	•		2	272 ST
Spyrogenes 238, SEQ		_	TA A. TT	AAT	• • • • • • • • • • • • • • • • • • • •	:		:	5	:	.A. TC. TO.	2	
Mavium 235.SEQ	£-	C	.TCC.CTCAA.CT	ANC	R	:	GAC	A.C.TA.A.CA.	A.A.G	g	8		
. Mtuberculosis 235.5EQ	F	GC.C.	.TCAA.C.	AAAC		:	GPC	A.C.T	์ ฮ์	8	8	C. 70.A.	
Ecoli 235.5EQ CCTAICTGCCGGGGGCCTGGAGACTGAGGGGGGCTCCTCCIACTACGAGGGCCCGGAGGACGCATCACTGGGGATTCCGGGTTCCCAATGCAATGCAATGGCACTGCCGGGAAAAAA	OCCUAR	1000110000	ACTION/CHON	D00000T	SCICCINGIA	CONGRACION	COCAGTOCACC	CATCACTOO	GTTCCCOTTG	TOTACC	MIGGGAC	TGCCCGGTM	
Kpneumoniae 235.5EQ	• • • • • • • • • • • • • • • • • • • •		+								:	:	2726
Kinfluenzae 238.820TA.		•	•	.TG.TTT						3	orc c. c. c	9	2732
Bhrochiseptica 238.520	•	• • • • • • • • • • • • • • • • • • • •		.T.CT ACA A.C	• • • • • • • • • • • • • • • • • • • •			J.O. J.			•		0 2689
Bparapartussis 238.820			.TT.CT.	T.CT ACA: . A.C	•			.T.C.T.			0	0	9 2691
Bpertussis 238.530	••••••		77.03	T.CT ACA A.C	• • • • • • • • • • • • • • • • • • • •			J.C. T.	A.C.	:		9	3690
Bospacia 238.520		:	TT.TTA	A				.A.C.T			c0TC.	C G	10 2701
Brallel 235.550	:	:	T ACTTA	A			••••••			0.0	.a.ca.cπca.	C Q	70 2704
Bpseudomallel 238.820	:		.T AUTT A	A	••••••			.A.C.T		0.0	.a.cπc.	C G	70 270
Ngonorrhoese 238.5EQA.	:	•	T MOTTC	O	•	•		A.C.T	٠.:			A 0	6 271
Neminigititedis 238.850			.T AOTTC			••••••		. A.C.T.		A.C.	A.C G. T TA G.	Α.Ο.Υ	2710
Paeruginose 238.SEQ			A T. T TT	A	•			A.C.T.		0		0	70 271
Vcholerae 238.58Q	• • • • • • • • • • • • • • • • • • • •		TAG.TA	A		•		A.C.T.		9	Que. T.		9012 0
Yenterocolitica 218.820	2		XX					×	>		>		2730

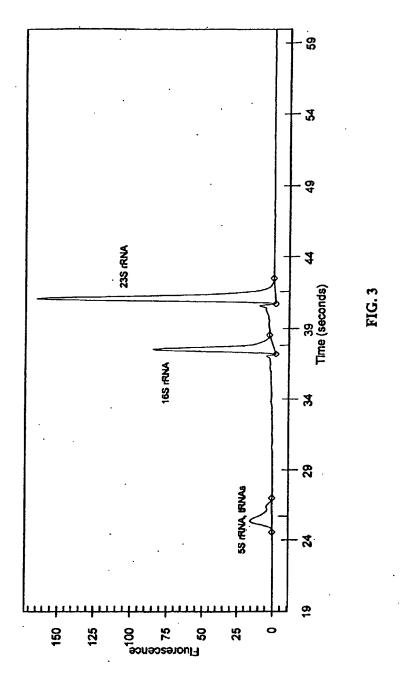
Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

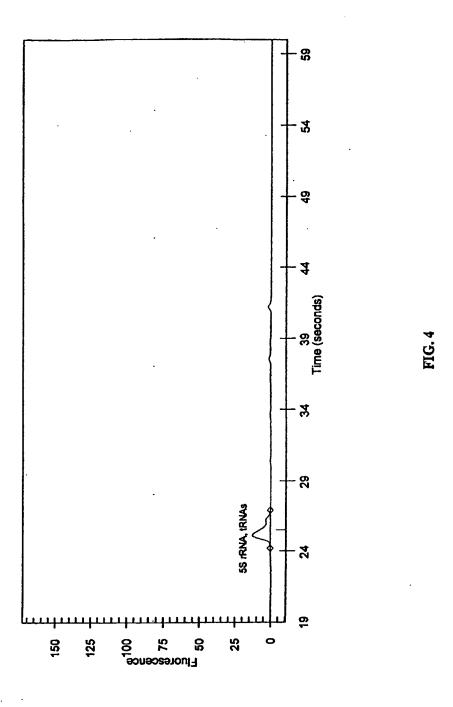
													ĺ
	2730 2740	2740	2750	2760	2770	2780	2790	2800	2810	2820	2830	2840	
Baubtilis 238.520	C.O.			T 0.000 T. A.	8	T. A. T.	1 :	ATT. GC-A.G. ANGT	7	ATC C 94	2	C4. E &	
_		• • • • • • • • • • • • • • • • • • • •		T0.00CT.A	8		AT.0.0	A. T. AT. 0.6A. OCTAGE	ATC	ATC C. GA. T.	, JC 6	5	286
a		, yc		TOT G. CCA T.A.	8		ATTT. T	ATTT. T A. CALAGT	ğ		4 0 DL		2856
Llactis 238.88Q			•	TG 0. CCA T.A.	8	:	A ATT.	ATT.G.A.GA-A.T	8	ACC CLOSE T	T. TCTG	44	2842
Imonocytogenes 238,580TCd	TCG			OT 0.000 T.A.	88:	:	T. TATTA. T	A.TATT.T COGNANGT	ATC	:	T. TC.G. A.	A FF	2876
Saureus 218,580	C.Q.	-	• • • • • • • • • • • • • • • • • • • •	T0.00T.A.	8	T.AA.T.	AA.T	AA.TCOOTTA-T	ATC	:	T. T. O A	TTC.NO.	2867
Smutens 238.880 Spneumonies 238.680	, AG . O. O.	y y		TOTG.CCCT.A.	G.000T.A.	:	AT.A.G	A.TAT.A.GC.OTTAGT	Ş		. A. AC.G. A.	4	2844
Spyogenes 238.820	, p			: :	4 £ 300 6		ATCATT	A.T. Angert At angerts	: :	Mac C take T TC G A	T. TC.0A		2846
Mavium 238.8EQ	4. Cdd	8			C.T.7	Ö	T AC- T	C. O. T. AC- T. GA. GAT-	: :				1007
Mtuberculosis 235.580 .TTCM	.TTCM	8		8	.C.T.7.	:	T. AC.	9 70	•	3			2002
Ecoli 238.88Q	TOCCOMPANANTA		AGRICATURACCATCTA	MOCACCANA	CTTCCC	NACOCCENANCTICOCCCENANTECTCCCTENCCTTIAN CONTECTIAN CONTENANCE CONTENANCE CONTENTED CON	TOCCTGACCC	TITAGOOTECT	A ADDRAG	COTTCAAACAC	ACCACCATICATA		2846
Kpneumoniae 218.520					:		3	Į,					2846
Hinfluensae 238.820	•							AOT.	•	OTTT.	4	£	2848
Bbrochiseptica 238.880.A	.A	8		8	. C. T. TCA.	TGA T G. A		03GMC.AG.TCCC	5	.gx	.0.	6	2808
Sparapertussis 238.520.A				8	.C.T. TGA	TGA T G. A.	:	0G-AC.AG.TCCC	5			E	2808
Bpertussis 235.880	. .			8	. C. T. TGB	TCA T G. A.	:	00-MC.MG.TOCC	8	8	C.Q		2807
Beepacia 138.820					ÿ	TTAA.A.	:	00GAC. AG. TCCC. T.	5	80c.a.			2821
BENETIES 238.880	F.	-			.с. Д	:		C. 00GMC G. TCCC. T.	:				2824
byseucomation 438.880 Tr.					.c. 11	:		.C.00GACG.TCCC.T.,	٠	8			2824
Monotthogod 430.084 .T		_		:	C. TCA.	:	30.52	13.638.G.		8	.c.g	gr	2833
Newton Street 435.00% Treet.				:		:	8.8	ACTTG.0GCC.CAA.					2834
Paginginoss 228.850	T	8		8	:	:	ogb	A GGAAC G. TTCC	•				2834
Venoterae 238.880	7 11				: 5	t	:	AG CT A GIT CG T. CB.	£ 4	J8	3	λ	2829
Yenterocolitice 238.SEGK	QK		••••••	RS	Υ	.Y	5	GAACTC	:			X	2849

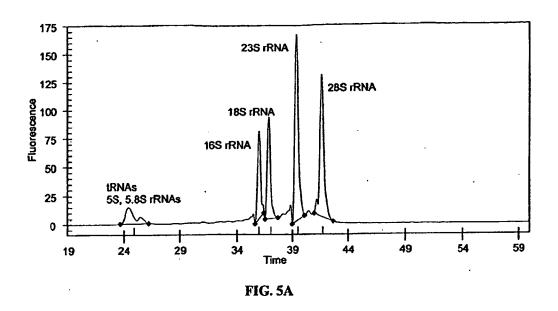
Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

				ŀ	l			
	2850	•	2860	2870		2880	2890	2000
	1			-				
BRUDCALIS 238.520		2	0.4.0.	9	A.A.	8	T XO. Y	.d T. TGA. C.R. A.G
Banthracis 238.650	0	ATG.T.	5	0	3	Ş	T AG. A	.GXIG.ICAIGGBAX
Efacaelis 238.5EQ	0.	4	MOT O.	8	*	8	7XG.A	.66CTTAOT.6COAACCGTAG.A.
Liactim 238.820	0	Ę	9.7.0	8	Ą	8	4 9Y-	.6h.TCh.T.6Cdh
. Imonocytogenes 238.580.6T.TA.A.GQQAAA	2.61		A.A.O.	8	3	8	T - AC A	A-A
Saureus 235.6EQ	0	ATG.T.	5	O	3	8	4 Y- 1	.0 ATG.TCATG
Smutans 238.SEQ	Ö	11.11	3	8	4	8	4 54	GTT.T.GhGhGGAA.
Spneumoniae 238.520	0	p	5	8	7.7	Ş	4 04	.0. T.10C. A.OTCO. TAX.
Spyogenes 238.820			A.A.Gr	8	3	ğ	T.T A.A. GT. GG. TAA. ACCTAG.A. T.	E
Mavium 239.520	0	77		8	C)	Č	.0TTAACCQQTCCTCG.	
Ktuberculesis 238.880 . G T Th G GT G	0	77	0	8	e.	8	AA A-	A A A C
Ecoli 238.5EQ	STACK	CONTRACT	ATCCCTTC	NO.	COOLE	TAATGA	GTAAGCCCAGCGATCCCTTCAACTAACCCCTAAATCAAACCCTTCAACTTTAAACCTTT	TENNO CHE
Kpneumonies 238.8EQ		,						
Hinfluensae 238.550		r.AT.T.	4.5		\$	ğ	T.AT.T. OT.A.	
Bbrochiseptica 235.520.d		A	¥		Y	ğ		Ú.
Sparapertusais 238.8EQ.G.TaTaA.	2.0.	7	¥		4	ğ		0.5
Bpercussis 238.5EQ	0	a	¥		4	ğ	.0	O.
Bospacia 235.520	:	1	0		4	ğ	D. T. D. T. S. T.	
Emallei 235.8EQ		ā	0		~	ğ	Q	4.0.T.0
Epseudomailei 218.5EQTA		ā	0		~	ğ	Ş	4 U.E.
Ngonorrhoese 218.620 .GG.Th.CGhCh.	0	9	£		ð	ğ		2
Neminigitatelle 238.820.0G.Th.CGhCh.		9	£		ฮ	P	Ş.	Ę.
Paeruginosa 235.520		13.4	0		4	P	TT.T G	4
Vcholerae 238.5BQ	:		0		f	2	201 T. C. T.	•
Yanterocolitica 215.550	8	9	2					
,				:	::::	::::		

Decoration 'Decoration #1': Hide (as '.') residues that match Ecoli 218.8EQ exactly.







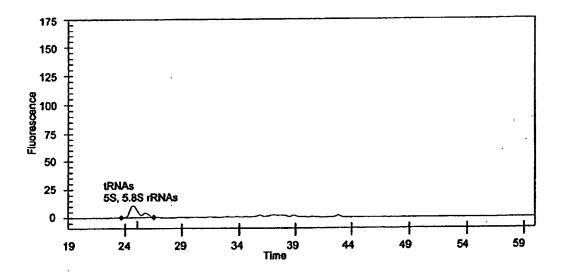


FIG. 5B

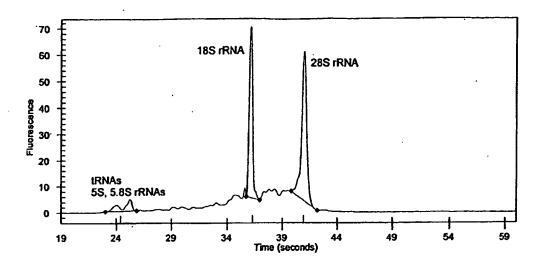


FIG. 6A

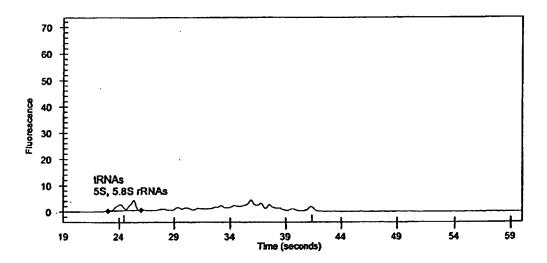


FIG. 6B

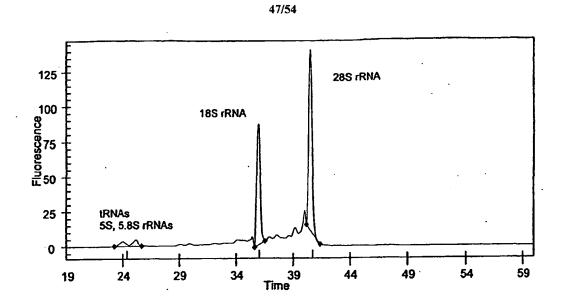


FIG. 7A

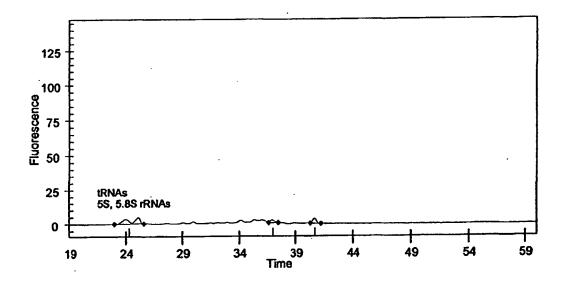


FIG. 7B

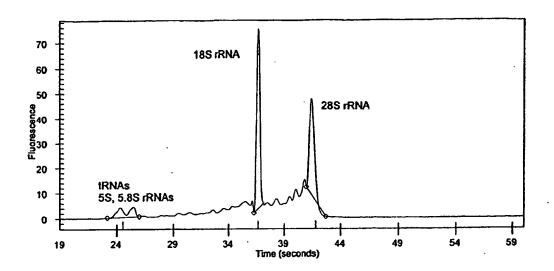


FIG. 8A

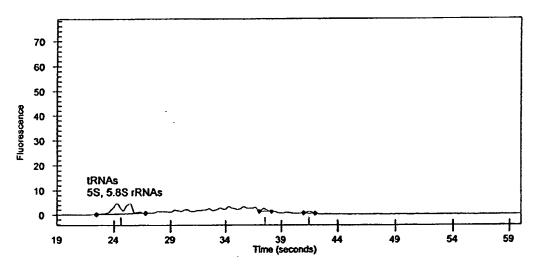


FIG. 8B

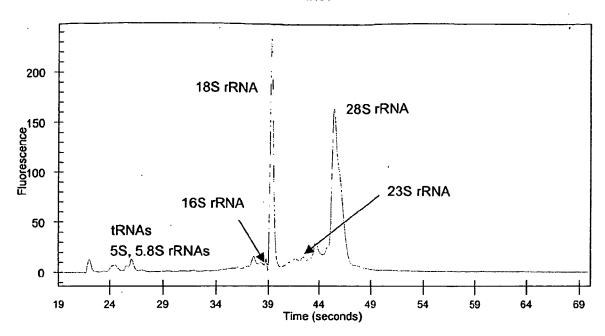


FIG. 9A

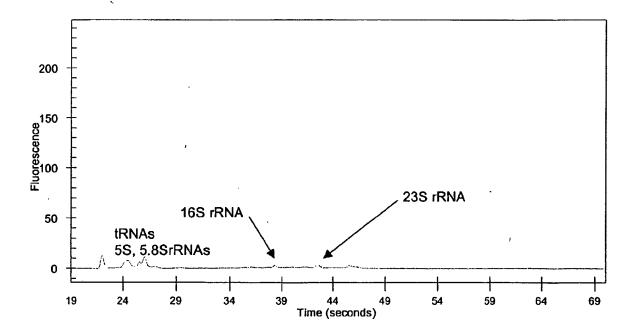
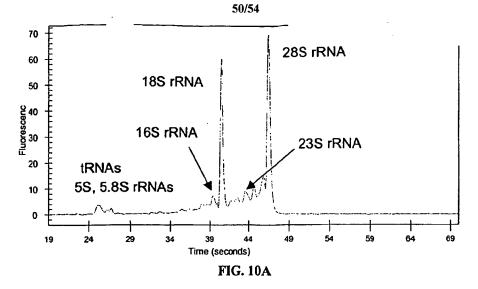
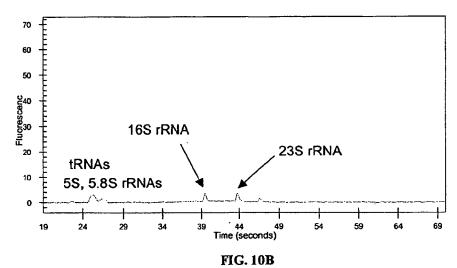


FIG. 9B

WO 03/054162 PCT/US02/41014





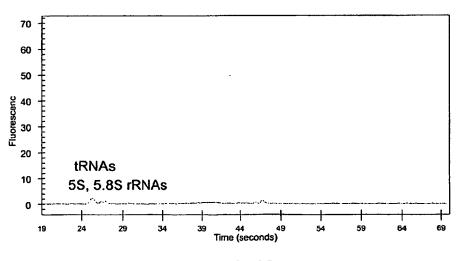


FIG. 10C

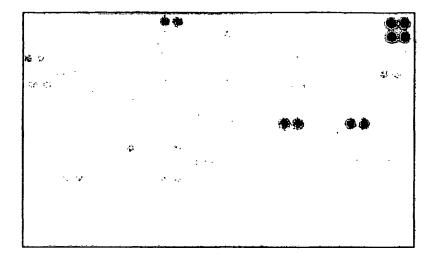


FIG. 11A



FIG. 11B

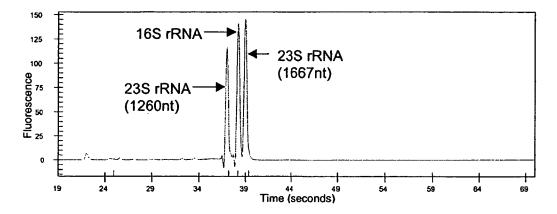


FIG. 12A

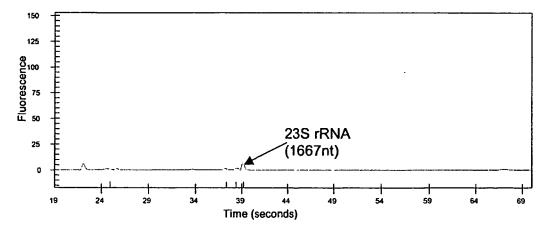


FIG. 12B

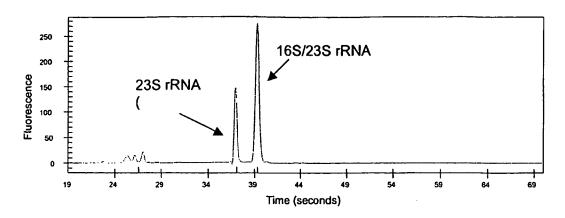


FIG. 13A

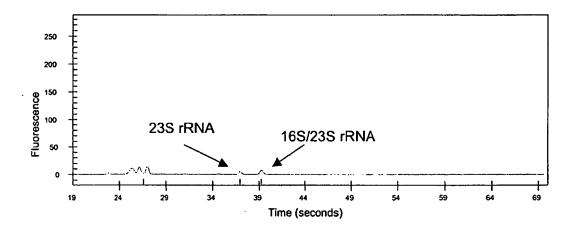


FIG. 13B

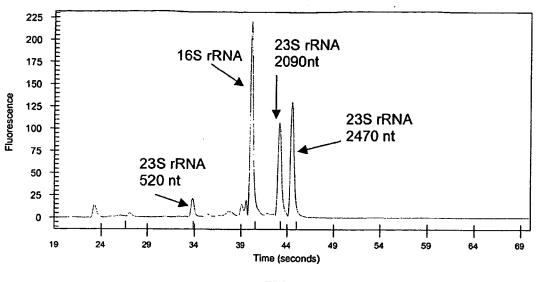


FIG 14A.

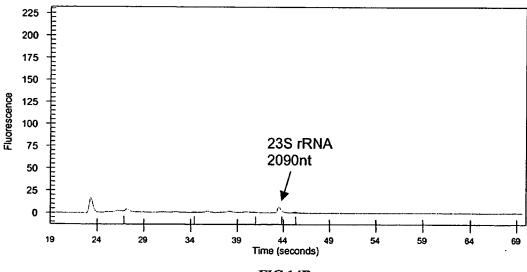


FIG 14B.

PCT/US02/41014 1/52

SEQUENCE LISTING

. .

```
<110> MURPHY, GEORGE L.
          WHITLEY, J. PENN
 5
     <120> METHOD AND SYSTEM FOR DEPLETING rRNA POPULATIONS
    <130> AMBI:076WO
10 <140> UNKNOWN
    <141> 2002-12-20
    <150> 10/029,397
    <151> 2001-12-20
15
    <160> 92
    <170> PatentIn Ver. 2.1
20 <210> 1
    <211> 22
    <212> DNA
    <213> Artificial Sequence
25 <220>
    <223> Description of Artificial Sequence: Synthetic
          Primer
    <400> 1
30 ctgctgcctc ccgtaggagt ct
                                                                      22
    <210> 2
    <211> 23
   <212> DNA
    <213> Artificial Sequence
    <223> Description of Artificial Sequence: Synthetic
40
    <400> 2
    cgtattaccg cggctgctgg cac
                                                                      23
45
    <210> 3
    <211> 23
    <212> DNA
    <213> Artificial Sequence
50
    <223> Description of Artificial Sequence: Synthetic
          Primer
55
    <400> 3
    cgcccagtaa ttccgattaa cgc
                                                                      23
```

	<210> 4	
	<211> 23	
5	<212> DNA	
	<213> Artificial Sequence	
	•	
	<220>	
	<223> Description of Artificial Sequence: Sy	nthetic
10	Primer	-
• •		
	<400> 4	
	tggactacca gggtatctaa tcc	23
	095000000 9550000000	
15		
••	<210> 5	
	<211> 23	
	<212> DNA	•
	<213> Artificial Sequence	
20	10.00	
	<220>	
	<223> Description of Artificial Sequence: Sy	nthetic
	Primer	
25	<400> 5	
	gggttgeget egttgeggga ett	23
	<210> 6	
30	<211> 23	
	<212> DNA	
	<213> Artificial Sequence	
	•	
	<220>	
35	<223> Description of Artificial Sequence: Sy	nthetic
	Primer	
	<400> 6	
	taaggaggtg atccaaccgc agg	23
40		
		•
	<210> 7	
	<211> 23	
	<212> DNA	
45	<213> Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Sy	nthetic
	Primer	
50		
	<400> 7	
	ggttcttttt cactcccctc gcc	23
55	<210> 8	
	<211> 23	

```
<212> DNA
     <213> Artificial Sequence
 5 <223> Description of Artificial Sequence: Synthetic
          Primer
    <400> 8
    gacccattat acaaaaggta cgc
                                                                       23
10
     <210> 9
     <211> 23
    <212> DNA
15 <213> Artificial Sequence
    <220>
     <223> Description of Artificial Sequence: Synthetic
          Primer
20
     <400> 9
                                                                       23
    gccccgttac atcttccgcg cag
25 <210> 10
    <211> 23
     <212> DNA
    <213> Artificial Sequence
30
     <223> Description of Artificial Sequence: Synthetic
          Primer
    <400> 10
35
                                                                       23
    cgacaaggaa tttcgctacc tta
     <210> 11
     <211> 22
40
    <212> DNA
    <213> Artificial Sequence
    <223> Description of Artificial Sequence: Synthetic
45
          Primer
     <400> 11
                                                                       22
    cttacccgac aaggaatttc gc
50
    <210> 12
    <211> 23
    <212> DNA
    <213> Artificial Sequence
55
```

<220>

	<223>	Description of Artificial Primer	Sequence:	Synthetic	
5	<400> gagcc	12 gacat cgaggtgcca aac			23
	<210>				
	<211>				
10	<212> <213>	DNA Artificial Sequence			
	<220>				
15	<223>	Description of Artificial Primer	Sequence:	Synthetic	
	<400>				
	ggttaa	agect caeggtteat t			21
20					
	<210>	14			
	<211>				
	<212>				
25	<213>	Artificial Sequence			
	<220>				
	<223>	Description of Artificial Primer	Sequence:	Synthetic	
30	<400>	14			
		gcac ggca			14
	<210>	16			
35	<211>				
,,	<212>				
		Artificial Sequence			
	<220>				
40		Description of Artificial	Sequence:	Synthetic	
		Primer	•	•	
	<400>	15			
45	cccctt	ctcc cgaagttacg ggg			23
	<210>	16			
	<211>				
	<212>				
50		Artificial Sequence			
•	<220>				
	<223>	Description of Artificial	Sequence:	Synthetic	
		Primer			
55	.465				
	<400>	16			

PCT/US02/41014

	gtgagctatt acgctttctt t		21
5	<210> 17 <211> 23		
_	<212> DNA <213> Artificial Sequence		
10	<220> <223> Description of Artificial Sequence:	Synthetic	
10	Primer	Synemetre	
	<400> 17		
1.5	taccggccgt gcgtacttag aca		23
15			
	<210> 18		
	<211> 23	•	
20	<212> DNA <213> Artificial Sequence		
20	(213) Altificial bequence		
	<220>		
	<223> Description of Artificial Sequence:	Synthetic	
	Primer		
25	.400. 10		
	<400> 18 tgccctccaa tggatcctcg tta		23
	igecologa iggalocity tia		23
30	<210> 19		
	<211> 23		
	<212> DNA		
	<213> Artificial Sequence		
35	<220>		
	<pre><223> Description of Artificial Sequence: Primer</pre>	Synthetic	
	<400> 19		
40	ctacggaaac cttgttacga ctt		23
	<210> 20		
15	<211> 23		
45	<212> DNA		
	<213> Artificial Sequence		
	<220>		
50	<223> Description of Artificial Sequence:	Synthetic	
50	Primer		
	<400> 20		
	gagcactggg cagaaatcac atc		23
55	-210- 21		
	<210> 21		

```
<211> 23
     <212> DNA
     <213> Artificial Sequence
5
     <220>
     <223> Description of Artificial Sequence: Synthetic
     <400> 21
10
    gtttcttttc ctccgctgac taa
                                                                        23
     <210> 22
     <211> 23
15
     <212> DNA
     <213> Artificial Sequence
     <223> Description of Artificial Sequence: Synthetic
20
           Primer
     <400> 22
                                                                        23
     tcctcagcca agcacataca cca
25
     <210> 23
     <211> 1427
     <212> DNA
     <213> Bacillus subtilis
30
     <220>
     <221> modified base
     <222> (554)..(873)
     \langle 223 \rangle N = A, C, G or T/U
35
     <400> 23
     qaqaqtttqa tcctqqctca ggacgaacgc tggcggcgtg cctaatacat gcaagtcgag 60
     cggacagatg ggagcttgct ccctgatgtt agcggcggac gggtgagtaa cacgtgggta 120
     acctgcctgt aagactggga taactccggg aaaccggggc taataccgga tggttgtttg 180
40
     aaccqcatqq ttcaaacata aaaggtggct tcggctacca cttacagatg gacccgcggc 240
     qcattaqcta gttggtgagg taacggctca ccaaggcaac gatgcgtagc cgacctgaga 300
     gggtgategg ceacactggg actgagacae ggeecagaet cetaegggag geageagtag 360
     ggaatettee geaatggaeg aaagtetgae ggageaaege egegtgagtg atgaaggttt 420
     teggategta aagetetgtt gttagggaag aacaagtace gttegaatag ggeggtacet 480
     tqacqqtacc taaccaqaaa qccacqqcta actacqtgcc agcagccgcg gtaatacgta 540
     qqtqqcaaqc qttntccqqa attattqggc gtaaaqggct cgcaggcggt ttcttaagtc 600
     tgatgtgaaa gcccccggct caaccgggga gggtcattgg aaactgggga acttgagtgc 660
     agaagaggag agtggaattc cacgtgtngc ggtgaaatgc gtagagatgt ggaggaacac 720
     cagtggcgaa ggcgactctc tggtctgtaa ctgacgctga ggagcgaaag cgtggggagc 780
50
     gaacaggatt agataccetg gtagtecacg cegtaaacga tgagtgetaa gtgttagggg 840
     gtttccgccc cttagtgctg cagtaacgca ttnagcactc cgcctgggga gtacggtcgc 900
     aaqactqaaa ctcaaaqgaa ttgacqgggg ccgcacaagc ggtggagcat gtggtttaat 960
     togaagcaac gogaagaacc ttaccaggto ttgacatoot otgacaatoo tagagatagg 1020
     acqtcttcqq qqqcaqaqtq acaqqtqqtq catqqttqtc qtcaqctcqt gtcqtgagat 1080
55
     gttgggttaa gtcccgcaac gagcgcaacc ctggatctta gttgccagca ttcagttggg 1140
```

cactctaagg tgactgccgg tgacaaaccg gaggaaggtg gggatgacgt caaatcatca 1200

```
tgccccttat gacctgggct acacacgtgc tacaatggac agaacaaagg gcagcgaaac 1260
     cgcgaggtta agccaatccc acaaatctgt tctcagttcg gatcgcagtc tgcaactcga 1320
     ctgcgtgaag ctggaatcgc tagtaatcgc ggatcagcat gccgcggtga atacgttccc 1380
     gggccttgta cacaccgccc gtcacaccac gagagtttgt aacaccc
                                                                       1427
     <210> 24
     <211> 1544
     <212> DNA
10
     <213> Bacillus anthracis
     <400> 24
     qtttgatcct ggctcaggat gaacgctggc ggcgtgccta atacatgcaa gtcgagcgaa 60
     tggattaaga gcttgctctt atgaagttag cggcggacgg gtgagtaaca cgtgggtaac 120
15
     ctgcccataa gactgggata actccgggaa accggggcta ataccggata acattttgaa 180
     ccgcatggtt cgaaattgaa aggcggcttc ggctgtcact tatggatgga cccgcgtcgc 240
     attagctagt tggtgaggta acggctcacc aaggcaacga tgcgtagccg acctgagagg 300
     gtgatcggcc acactgggac tgagacacgg cccagactcc tacgggaggc agcagtaggg 360
     aatcttccgc aatggacgaa agtctgacgg agcaacgccg cgtgagtgat gaaggctttc 420
20
    gggtcgtaaa actctgttgt tagggaagaa caagtgctag ttgaataagc tggcaccttg 480
     acggtaccta accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg 540
     tggcaagcgt tatccggaat tattgggcgt aaagcgcgcg caggtggttt cttaagtctg 600
     atgtgaaagc ccacggctca accgtggagg gtcattggaa actgggagac ttgagtgcag 660
     aagaggaaag tggaattcca tgtgtagcgg tgaaatgcgt agagatatgg aggaacacca 720
25
     gtggcgaagg cgactttctg gtctgtaact gacactgagg cgcgaaagcg tggggagcaa 780
     acaggattag ataccetggt agtecacgce gtaaacgatg agtgctaagt gttagagggt 840
     ttccgccctt tagtgctgaa gttaacgcat taagcactcc gcctggggag tacggccgca 900
     aggetgaaac teaaaggaat tgaeggggge eegeacaage ggtggageat gtggtttaat 960
     tcgaagcaac gcgaagaacc ttaccaggtc ttgacatcct ctgacaaccc tagagatagg 1020
     getteteett egggageaga gtgacaggtg gtgcatggtt gtegteaget egtgtegtga 1080
    gatgttgggt taagtcccgc aacgagcgca acccttgatc ttagttgcca tcattaagtt 1140
    gggcacteta aggtgactge cggtgacaaa ccggaggaag gtggggatga cgtcaaatca 1200
     tcatgcccct tatgacctgg gctacacacg tgctacaatg gacggtacaa agagctgcaa 1260
    gaccgcgagg tggagctaat ctcataaaac cgttctcagt tcggattgta ggctgcaact 1320
35
    cgcctacatg aagctggaat cgctagtaat cgcggatcag catgccgcgg tgaatacgtt 1380
     cccgggcctt gtacacaccg cccgtcacac cacgagagtt tgtaacaccc gaagtcggtg 1440
     gggtaacctt tttggagcca gccgcctaag gtgggacaga tgattggggt gaagtcgtaa 1500
     caaggtagec gtateggaag gtgeggetgg ateaecteet ttet
40
     <210> 25
     <211> 1449
     <212> DNA
     <213> Enterococcus faecalis
45
     <400> 25
     cqaacqctqq cqqcqtqcct aatacatqca agtcqaacqc ttctttcctc ccqaqtqctt 60
     gcactcaatt ggaaagagga gtggcggacg ggtgagtaac acgtgggtaa cctacccatc 120
     agagggggat aacacttgga aacaggtgct aataccgcat aacagtttat gccgcatggc 180
50
     ataagagtga aaggcgcttt cgggtgtcgc tgatggatgg acccgcggtg cattagctag 240
     ttggtgaggt aacggctcac caaggccacg atgcatagcc gacctgagag ggtgatcggc 300
     cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtagg gaatcttcgg 360
    caatggacga aagtctgacc gagcaacgcc gcgtgagtga agaaggtttt cggatcgtaa 420
     aactctgttg ttagagaaga acaaggacgt tagtaactga acgtcccctg acggtatcta 480
55
     accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg tggcaagcgt 540
     tgtccggatt tattgggcgt aaagcgagcg caggcggttt cttaagtctg atgtgaaagc 600
```

```
ccccggctca accggggagg gtcattggaa actgggagac ttgagtgcag aagaggagag 660
     tggaattcca tgtgtagcgg tgaaatgcgt agatatatgg aggaacacca gtggcgaagg 720
     cggctctctg gtctgtaact gacgctgagg ctcgaaagcg tggggagcaa acaggattag 780
     ataccctggt agtccacgcc gtaaacgatg agtgctaagt gttggagggt ttccgccctt 840
     cagtgctgca gcaaacgcat taagcactcc gcctggggag tacgaccgca aggttgaaac 900
     tcaaaggaat tgacggggc ccgcacaagc ggtggagcat gtggtttaat tcgaagcaac 960
     gcgaagaacc ttaccaggtc ttgacatcct ttgaccactc tagagataga gctttccctt 1020
     cggggacaaa gtgacaggtg gtgcatggtt gtcgtcagct cgtgtcgtga gatgttgggt 1080
     taagtcccgc aacgagcgca accettattg ttagttgcca tcatttagtt gggcactcta 1140
10
    gcgagactgc cggtgacaaa ccggaggaag gtggggatga cgtcaaatca tcatgcccct 1200
     tatgacetgg getacacacg tgetacaatg ggaagtacaa egagtegeta gacegegagg 1260
     tcatgcaaat ctcttaaagc ttctctcagt tcggattgca ggctgcaact cgcctgcatg 1320
     aagccggaat cgctagtaat cgcggatcag cacgccgcgg tgaatacgtt cccgggcctt 1380
     gtacacaccg cccgtcacac cacgagagtt tgtaacaccc gaagteggtg aggtaacctt 1440
15
     tttggagcc
     <210> 26
     <211> 1548
20
    <212> DNA
     <213> Lactococcus lactis
     <400> 26
     tttatttgag agtttgatcc tggctcagga cgaacgctgg cggcgtgcct aatacatgca 60
    agttgagcgc tgaaggttgg tacttgtacc gactggatga gcagcgaacg ggtgagtaac 120
    gcgtggggaa tctgcctttg agcgggggac aacatttgga aacgaatgct aataccgcat 180
    aaaaacttta aacacaagtt ttaagtttga aagatgcaat tgcatcactc aaagatgatc 240
    ccgcgttgta ttagctagtt ggtgaggtaa aggctcacca aggcgatgat acatagccga 300
    cctgagaggg tgatcggcca cattgggact gagacacggc ccaaactcct acgggaggca 360
30
    gcagtaggga atcttcggca atggacgaaa gtctgaccga gcaacgccgc gtgagtgaag 420
    aaggttttcg gatcgtaaaa ctctgttggt agagaagaac gttggtgaga gtggaaagct 480
    catcaagtga cggtaactac ccagaaaggg acggctaact acgtgccagc agccgcggta 540
    atacgtaggt cccgagcgtt gtccggattt attgggcgta aagcgagcgc aggtggttta 600
    ttaagtctgg tgtaaaaggc agtggctcaa ccattgtatg cattggaaac tggtagactt 660
    gagtgcagga gaggagagtg gaattccatg tgtagcggtg aaatgcgtag atatatggag 720
    gaacaceggt ggcgaaagcg gctctctggc ctgtaactga cactgaggct cgaaagcgtg 780
    gggagcaaac aggattagat accetggtag tecaegeegt aaacgatgag tgetagatgt 840
     agggagetat aagttetetg tategeaget aaegeaataa geaeteegee tggggagtae 900
    gaccgcaagg ttgaaactca aaggaattga cgggggcccg cacaagcggt ggagcatgtg 960
40
    gtttaattcg aagcaacgcg aagaacctta ccaggtcttg acatactcgt gctattccta 1020
    gagataggaa gttccttcgg gacacgggat acaggtggtg catggttgtc gtcagctcgt 1080
    gtcgtgagat gttgggttaa gtcccgcaac gagcgcaacc cctattgtta gttgccatca 1140
    ttaagttggg cactctaacg agactgccgg tgataaaccg gaggaaggtg gggatgacgt 1200
    caaatcatca tgccccttat gacctgggct acacacgtgc tacaatggat ggtacaacga 1260
    gtcgcgagac agtgatgttt agctaatctc ttaaaaccat tctcagttcg gattgtaggc 1320
    tgcaactcgc ctacatgaag tcggaatcgc tagtaatcgc ggatcagcac gccgcggtga 1380
    atacgttccc gggccttgta cacaccgccc gtcacaccac gggagttggg agtacccgaa 1440
    gtaggttgcc taaccgcaag gagggcgctt cctaaggtaa gaccgatgac tggggtgaag 1500
    tcgtaacaag gtagccgtat cggaaggtgc ggctggatca cctccttt
50
    <210> 27
    <211> 1524
    <212> DNA
55
```

<213> Listeria monocytogenes

```
<400> 27
     gcctgcaggt cgacaacaga gtttgatcat ggctcaggac gaacgctggc ggcgtgccta 60
     atacatgcaa gtcgaacgaa cggaggaaga gcttgctctt ccaaagttag tggcggacgg 120
     gtgagtaaca cgtgggcaac ctgcctgtaa gttggggata actccgggaa accggggcta 180
     ataccgaatg ataaagtgtg gcgcatgcca cgcttttgaa agatggtttc ggctatcgct 240
     tacagatggg cccgcggtgc attagctagt tggtagggta atggcctacc aaggcaacga 300
     tgcatagccg acctgagagg gtgatcggcc acactgggac tgagacacgg cccagactcc 360
     tacgggaggc agcagtaggg aatcttccgc aatggacgaa agtctgacgg agcaacgccg 420
     cgtgtatgaa gaaggttttc ggatcgtaaa gtactgttgt tagagaagaa caaggataag 480
10
     agtaactgct tgtcccttga cggtatctaa ccagaaagcc acggctaact acgtgccagc 540
     agccgcggta atacgtaggt ggcaagcgtt gtccggattt attgggcgta aagcgcgcgc 600
     aggoggtott ttaagtotga tgtgaaagco cooggottaa coggggaggg toattggaaa 660
     ctggaagact ggagtgcaga agaggagat ggaattccac gtgtagcggt gaaatgcgta 720
     gatatgtgga ggaacaccag tggcgaaggc gactctctgg tctgtaactg acgctgaggc 780
15
     gcgaaagcgt ggggagcaaa caggattaga taccctggta gtccacgccg taaacgatga 840
     gtgctaagtg ttagggggtt tccgcccctt agtgctgcag ctaacgcatt aagcactctg 900
     cctggggagt acgaccgcaa ggttgaaact caaaggaatt gacgggggcc cgcacaagcg 960
     tggagcatgt ggtttaattc gaagcaacgc gaagaacctt accaggtctt gacatccttt 1020
     gaccactetg gagacagage tttecetteg ggacaaagtg acaggtggtg catggttgte 1080
20
     gtcagctcgt gtcgtgagat gttgggttaa gtcccgcaac gagcgcaacc cttgatttta 1140
     gttgccagca tttagttggg cactctaaag tgactgccgg tgcaagccga ggaaggtggg 1200
     gatgacgtca aatcatcatg ccccttatga cctgggctac acacgtgcta caatggatag 1260
     tacaaagggt cgcgaagccg cgaggtggag ctaatcccat aaaactattc tcagttcgga 1320
     ttgtaggctg caactcgcct acatgaagcc ggaatcgcta gtaatcgtgg atcagcatgc 1380
25
     cacggtgagt acgttcccgg gccttgtaca caccgcccgt cacaccacga gagtttgtaa 1440
     cacccgaagt cggtagggta acctttatgg agccagccgc cgaaggtggg acagataatt 1500
     ggggtgaagt cgtaacaagg taaa
                                                                       1524
30
     <210> 28
     <211> 1555
     <212> DNA
     <213> Staphylococcus aureus
35
     <400> 28
     ttttatggag agtttgatcc tggctcagga tgaacgctgg cggcgtgcct aatacatgca 60
     agtcgagcga acggacgaga agcttgcttc tctgatgtta gcggcggacg ggtgagtaac 120
     acgtggataa cctacctata agactgggat aacttcggga aaccggagct aataccggat 180
     aatattttga accgcatggt tcaaaagtga aagacggtct tgctgtcact tatagatgga 240
40
     teegegetge attagetagt tggtaaggta aeggettace aaggeaaega taegtageeg 300
     acctgagagg gtgatcggcc acactggaac tgagacacgg tccagactcc tacgggaggc 360
     agcagtaggg aatcttccgc aatgggcgaa agcctgacgg agcaacgccg cgtgagtgat 420
     gaaggtette ggategtaaa actetgttat tagggaagaa catatgtgta agtaactgtg 480
     cacatettga eggtacetaa teagaaagee aeggetaaet aegtgeeage ageegeggta 540
45
     atacgtaggt ggcaagcgtt atccggaatt attgggcgta aagcgcgcgt aggcggtttt 600
     ttaagtetga tgtgaaagee caeggeteaa eegtggaggg teattggaaa etggaaaaet 660
     tgagtgcaga agaggaaagt ggaattccat gtgtagcggt gaaatgcgca gagatatgga 720
    ggaacaccag tggcgaaggc gactttctgg tctgtaactg acgctgatgt gcgaaagcgt 780
     ggggatcaaa caggattaga taccctggta gtccacgccg taaacgatga gtgctaagtg 840
50
    ttagggggtt tccgcccctt agtgctgcag ctaacgcatt aagcactccg cctggggagt 900
    acgaccgcaa ggttgaaact caaaggaatt gacggggacc cgcacaagcg gtggagcatg 960
    tggtttaatt cgaagcaacg cgaagaacct taccaaatct tgacatcctt tgacaactct 1020
    agagatagag ccttcccctt cgggggacaa agtgacaggt ggtgcatggt tgtcgtcagc 1080
    tegtgtegtg agatgttggg ttaagteegg caacgagege aaccettaag ettagttgee 1140
55
    atcattaagt tgggcactct aagttgactg ccggtgacaa accggaggaa ggtggggatg 1200
    acqtcaaatc atcatgcccc ttatgatttg ggctacacac gtgctacaat ggacaataca 1260
```

aagggcagcg aaaccgcgag gtcaagcaaa teccataaag ttgttetcag ttcggattgt 1320 agtetgeaac tegactacat gaagetggaa tegetagtaa tegtagatea geatgetaeg 1380 gtgaatacgt tcccgggtat tgtacacacc gcccgtcaca ccacgagagt ttgtaacacc 1440 cgaagccggt ggagtaacct tttaggagct agccgtcgaa ggtgggacaa atgattgggg 1500 tgaagtcgta acaaggtagc cgtatcggaa ggtgcggctg gatcacctcc tttct <210> 29 <211> 1551 10 <212> DNA <213> Streptococcus mutans <400> 29 agagtttgat cctggctcag gacgaacgct ggcggcgtgc ctaatacatg caagtgggac 60 gcaaggaaac acactgtgct tgcacaccgt gttttcttga gtcgcgaacg ggtgagtaac 120 gcgtaggtaa cctgcctatt agcgggggat aactattgga aacgatagct aataccgcat 180 aatattaatt attgcatgat aattgattga aagatgcaag cgcatcacta gtagatggac 240 ctgcgttgta ttagctagtt ggtaaggtaa gagcttacca aggcgacgat acatagccga 300 cctgagaggg tgatcggcca cactgggact gagacacggc ccagactcct acgggaggca 360 20 gcagtaggga atcttcggca atggacgaaa gtctgaccga gcaacgccgc gtgagtgaag 420 aaggttttcg gatcgtaaag ctctgttgta agtcaagaac gtgtgtgaga gtggaaagtt 480 cacacagtga cggtagctta ccagaaaggg acggctaact acgtgccagc agccgcggta 540 atacgtaggt cccgagcgtt gtccggattt attgggcgta aagggagcgc aggcggtcag 600 gaaagtctgg agtaaaaggc tatggctcaa ccatagtgtg ctctggaaac tgtctgactt 660 gagtgcagaa ggggagagtg gaattccatg tgtagcggtg aaatgcgtag atatatggag 720 gaacaccagt ggcgaaagcg gctctctggt ctgtcactga cgctgaggct cgaaagcgtg 780 ggtagcgaac aggattagat accetggtag tecaegeegt aaacgatgag tgctaggtgt 840 taggecettt ceggggetta gtgceggage taacgcaata agcacteege etggggagta 900 cgaccgcaag gttgaaactc aaaggaattg acgggggccc gcacaagcgg tggagcatgt 960 30 ggtttaattc gaagcaacgc gaagaacctt accaggtctt gacatcccga tgctattctt 1020 agagatagga agttacttcg gtacatcgga gacaggtggt gcatggttgt cgtcagctcg 1080 tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac ccttattgtt agttgccatc 1140 attaagttgg gcactctagc gagactgccg gtaataaacc ggaggaaggt ggggatgacg 1200 tcaaatcatc atgcccctta tgacctgggc tacacagtg ctacaatggt cggtacaacg 1260 agttgcgagc cggtgacggc aagctaatct ctgaaagccg atctcagttc ggattggagg 1320 ctgcaactcg cctccatgaa gtcggaatcg ctagtaatcg cggatcagca cgccgcggtg 1380 aatacgttcc cgggccttgt acacaccgcc cgtcacacca cgagagtttg taacacccga 1440 agtcggtgag gtaacctttt aagggccaag ccgcctaagg tgggatggat gattggggtg 1500 aagtcgtaac aaggtagccg tatcggaagg tgcggctgga tcacctcctt t 40 <210> 30 <211> 1515 <212> DNA 45 <213> Streptococcus pneumoniae <400> 30 atttgateet ggeteaggae gaaegetgge ggegtgeeta atacatgeaa gtagaaeget 60 gaaggaggag cttgcttctc tggatgagtt gcgaacgggt gagtaacgcg taggtaacct 120 50 gcctggtagc gggggataac tattggaaac gatagctaat accgcataag agtggatgtt 180 gcatgacatt tgcttaaaag gtgcacttgc atcactacca gatggacctg cgttgtatta 240 gctagttggt ggggtaacgg ctcaccaagg cgacgataca tagccgacct gagagggtga 300 tcggccacac tgggactgag acacgkccca gactcctacg ggaggcagca gtagggaatc 360 tteggeaatg gacggaagte tgaccgagea acgccgegtg agtgaagaag gtttteggat 420

cgtaaagctc tgttgtaaga gaagaacgag tgtgagagtg gaaagttcac actgtgacgg 480 tatcttacca gaaagggacg gctaactacg tgccagcagc cgcggtaata cgtaggtccc 540

55

gagcgttgtc cggatttatt gggcgtaaag cgagcgcagg cggttagata agtctgaagt 600 taaaggctgt ggcttaacca tagtaggctt tggaaactgt ttaacttgag tgcaagaggg 660 gagagtggaa ttccatgtgt agcggtgaaa tgcgtagata tatggaggaa caccggtggc 720 gaaagegget etetggettg taactgaege tgaggetega aagegtgggg ageaaacagg 780 attagatace etggtagtee acgetgtaaa egatgagtge taggtgttag accettteeg 840 gggtttagtg ccgtagctaa cgcattaagc actccgcctg gggagtacga ccgcaaggtt 900 gaaactcaaa ggaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcgaa 960 gcaacgcgaa gaaccttacc aggtcttgac atccctctga ccgctctaga gatagagttt 1020 tccttcggga cagaggtgac aggtggtgca tggttgtcgt cagctcgtgt cgtgagatgt 1080 10 tgggttaagt cccgcaacga gcgcaacccc tattgttagt tgccatcatt cagttgggca 1140 ctctagcgag actgccggta ataaaccgga ggaaggtggg gatgacgtca aatcatcatg 1200 ccccttatga cctgggctac acacgtgcta caatggctgg tacaacgagt cgcaagccgg 1260 tgacggcaag ctaatctctt aaagccagtc tcagttcgga ttgtaggctg caactcgcct 1320 acatgaagtc ggaatcgcta gtaatcgcgg atcagcacgc cgcggtgaat acgttcccgg 1380 15 gccttgtaca caccgcccgt cacaccacga gagtttgtaa cacccgaagt cggtgaggta 1440 accgtaagga gccagccgcc taaggtggga tagatgattg gggtgaagtc gtaacaaggt 1500 cagccgtttg ggaga 20 <210> 31 <211> 1335 <212> DNA <213> Streptococcus pyogenes 25 <400> 31 gaacgggtga gtaacgcgta ggtaacctac ctcatagcgg gggataacta ttggaaacga 60 tagctaatac cgcataagag agactaacgc atgttagtaa tttaaaaggg gcaattgctc 120 cactatgaga tggacctgcg ttgtattagc tagttggtga ggtaaaggct caccaaggcg 180 acgatacata gccgacctga gagggtgatc ggccacactg ggactgagac acggcccaga 240 30 ctcctacggg aggcagcagt agggaatett eggcaatggg ggcaaccetg acegagcaac 300 gccgcgtgag tgaagaaggt tttcggatcg taaagctctg ttgttagaga agaatgatgg 360 tgggagtgga aaatccacca agtgacggta actaaccaga aagggacggc taactacgtg 420 ccagcagceg cggtaatacg taggtcccga gcgttgtccg gatttattgg gcgtaaagcg 480 agcgcaggcg gttttttaag tctgaagtta aaggcattgg ctcaaccaat gtacgctttg 540 35 gaaactggag aacttgagtg cagaagggga gagtggaatt ccatgtgtag cggtgaaatg 600 cgtagatata tggaggaaca ccggtggcga aagcggctct ctggtctgta actgacgctg 660 aggetegaaa gegtggggag caaacaggat tagataccet ggtagteeac geegtaaacg 720 atgagtgcta ggtgttaggc cctttccggg gcttagtgcc ggagctaacg cattaagcac 780 tecgeetggg gagtaegace geaaggttga aacteaaagg aattgaeggg ggeeegeaca 840 40 agcggtggag catgtggttt aattcgaagc aacgcgaaga accttaccag gtcttgacat 900 cccgatgccc gctctagaga tagagtttta cttcggtaca tcggtgacag gtggtgcatg 960 gttgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc cgcaacgagc gcaaccccta 1020 ttgttagttg ccatcattaa gttgggcact ctagcgagac tgccggtaat aaaccggagg 1080 aaggtgggga tgacgtcaaa tcatcatgcc ccttatgacc tgggctacac acgtgctaca 1140 45 atggttggta caacgagtcg caagccggtg acggcaagct aatctcttaa agccaatctc 1200 aqtteggatt qtaggetgea actegeetae atgaagtegg aategetagt aategeggat 1260 caqcacqccq cggtgaatac gttcccgggc cttgtacaca ccgcccgtca caccacgaga 1320 gtttgtaaca cccga 50 <210> 32 <211> 1465 <212> DNA <213> Mycobacterium avium 55

<220>

WO 03/054162 PCT/US02/41014

```
<221> modified_base
    <222> (298)..(881)
    <223> N = A, C, G or T/U
    <400> 32
    ggcggcgtgc ttaacacatg caagtcgaac ggaaaggcct cttcggaggt actcgagtgg 60
    cgaacgggtg agtaacacgt gggcaatcta ccctgcactt cgggataagc ctgggaaact 120
    gggtctaata ccggatagga cctcaagacg catgtcttct ggtggaaagc ttttgcggtg 180
    tgggatggc ccgcggccta tcagcttgtt ggtggggtga cggcctacca aggcgacgac 240
    qqqtaqccgq cctgagaggg tgtccggcca cactgggact gagatacggc ccagactnct 300
10
    acgggaggca gcagtgggga atattgcaca atgggcgcaa gcctgatgca gcgacgccgc 360
    gtgggggatg acggccttcg ggttgtaaac ctctttcacc atcgacgaag gtccgggttt 420
    tctcggattg acggtaggtg gagaagaagc accggccaac tacgtgccag cagccgcggt 480
    aatacgtagg gtgcgagcgt tgtccggaat tactgggcgt aaagagctcg taggtggttt 540
15
    gtcgcgttgt tcgtgaaatc tcacggctta actgtgagcg tgcgngcgat acgggcagac 600
    tagagtactg caggggagac tggaattcct ggtgtagcgg tggaatgcgc agatatcagg 660
    aggaacaccg gtggcgaagg cgggtctctg ggcagtaact gacgctgagg agcgaaagcg 720
    tggggagcga acaggattag ataccetggt agtecacgne gtaaacggtg ggtactaggt 780
    gtgggtttcc ttccttggga tccgtgccgt agctaacgca ttaagtaccc cgcctgggga 840
20
    gtacggncgc aaggctaaaa ctcaaaggaa ttgacggggg nccgcacaag cggcggagca 900
     tqtqqattaa ttcqatgcaa cgcgaagaac cttacctggg tttgacatgc acaggacgcg 960
    tctagagata ggcgttccct tgtggcctgt gtgcaggtgg tgcatggctg tcgtcagctc 1020
    gtgtcgtgag atgttgggtt aagtcccgca acgagcgcaa cccttgtctc atgttgccag 1080
    cgggtaatgc cggggactcg tgagagactg ccggggtcaa ctcggaggaa ggtggggatg 1140
25
    acqtcaaqtc atcatgcccc ttatgtccag ggcttcacac atgctacaat ggccggtaca 1200
    aagggctgcg atgccgtaag gttaagcgaa toottttaaa gccggtctca gttcggattg 1260
    gggtctgcaa ctcgaccca tgaagtcgga gtcgctagta atcgcagatc agcaacgctg 1320
    cggtgaatac gttcccgggc cttgtacaca ccgcccgtca cgtcatgaaa gtcggtaaca 1380
    cccgaagcca gtggcctaac ccttttggga gggagctgtc gaaggtggga tcggcgattg 1440
30
    ggacgaagtc gtaacaaggt agccg
     <210> 33
    <211> 1536
35
     <212> DNA
     <213> Mycobacterium tuberculosis
     <400> 33
    tttgtttgga gagtttgatc ctggctcagg acgaacgctg gcggcgtgct taacacatgc 60
40
     aagtcgaacg gaaaggtctc ttcggagata ctcgagtggc gaacgggtga gtaacacgtg 120
    ggtgatctgc cctgcacttc gggataagcc tgggaaactg ggtctaatac cggataggac 180
     cacgggatgc atgtcttgtg gtggaaagcg ctttagcggt gtgggatgag cccgcggcct 240
     atcagettgt tggtggggtg aeggeetace aaggegaega egggtageeg geetgagagg 300
    qtqtccqqcc acactqqqac tqaqatacqq cccaqactcc tacqqqaqqc agcaqtqqqq 360
45
     aatattgcac aatgggcgca agcctgatgc agcgacgccg cgtgggggat gacggccttc 420
     gggttgtaaa cetettteae categaegaa ggteegggtt eteteggatt gaeggtaggt 480
     ggagaagaag caccggccaa ctacgtgcca gcagccgcgg taatacgtag ggtgcgagcg 540
     ttgtccggaa ttactgggcg taaagagctc gtaggtggtt tgtcgcgttg ttcgtgaaat 600
     ctcacggctt aactgtgagc gtgcgggcga tacgggcaga ctagagtact gcaggggaga 660
50
     ctggaattcc tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtggcgaag 720
    gegggtetet gggeagtaac tgaegetgag gagegaaage gtggggageg aacaggatta 780
    gataccctgg tagtccacgc cgtaaacggt gggtactagg tgtgggtttc cttccttggg 840
    atccgtgccg tagctaacgc attaagtacc ccgcctgggg agtacggccg caaggctaaa 900
     actcaaagga attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca 960
55
     acgcgaagaa ccttacctgg gtttgacatg cacaggacgc gtctagagat aggcgttccc 1020
     ttgtggcctg tgtgcaggtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt 1080
```

```
taagtcccgc aacgagcgca accettgtct catgttgcca gcacgtaatg gtggggactc 1140
    qtgaqagact gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc 1200
    cttatgtcca gggcttcaca catgctacaa tggccggtac aaagggctgc gatgccgcga 1260
    ggttaagcga atoottaaaa googgtotoa gttoggatog gggtotgcaa otogaccoog 1320
    tgaagtcgga gtcgctagta atcgcagatc agcaacgctg cggtgaatac gttcccgggc 1380
    cttgtacaca ccgcccgtca cgtcatgaaa gtcggtaaca cccgaagcca gtggcctaac 1440
    cctcgggagg gagctgtcga aggtgggatc ggcgattggg acgaagtcgt aacaaggtag 1500
    ccgtaccgga aggtgcggct ggatcacctc ctttct
10
    <210> 34
    <211> 1536
    <212> DNA
     <213> Escherichia coli
15
    <400> 34
    tttgtttgga gagtttgatc ctggctcagg acgaacgctg gcggcgtgct taacacatgc 60
    aagtcgaacg gaaaggtctc ttcggagata ctcgagtggc gaacgggtga gtaacacgtg 120
    ggtgatctgc cctgcacttc gggataagcc tgggaaactg ggtctaatac cggataggac 180
20
    cacgggatgc atgtcttgtg gtggaaagcg ctttagcggt gtgggatgag cccgcggcct 240
    atcagettgt tggtggggtg aeggeetaec aaggegaega egggtageeg geetgagagg 300
    gtgtccggcc acactgggac tgagatacgg cccagactcc tacgggaggc agcagtgggg 360
    aatattgcac aatgggcgca agcctgatgc agcgacgccg cgtggggggat gacggccttc 420
    gggttgtaaa cctctttcac catcgacgaa ggtccgggtt ctctcggatt gacggtaggt 480
25
    ggagaagaag caccggccaa ctacgtgcca gcagccgcgg taatacgtag ggtgcgagcg 540
    ttgtccggaa ttactgggcg taaagagctc gtaggtggtt tgtcgcgttg ttcgtgaaat 600
    ctcacggctt aactgtgagc gtgcgggcga tacgggcaga ctagagtact gcaggggaga 660
    ctggaattcc tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtggcgaag 720
    gegggtetet gggeagtaac tgaegetgag gagegaaage gtggggageg aacaggatta 780
    gataccetgg tagtecacge egtaaaeggt gggtactagg tgtgggttte ettecttggg 840
    atcogtgccg tagctaacgc attaagtacc ccgcctgggg agtacggccg caaggctaaa 900
    actcaaagga attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca 960
    acgcgaagaa cettacetgg gtttgacatg cacaggacge gtctagagat aggcgttece 1020
    ttgtggcctg tgtgcaggtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt 1080
35
    taagtcccgc aacgagcgca accettgtct catgttgcca gcacgtaatg gtggggactc 1140
    gtgagagact gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc 1200
    cttatgtcca gggcttcaca catgctacaa tggccggtac aaagggctgc gatgccgcga 1260
    ggttaagcga atccttaaaa gccggtctca gttcggatcg gggtctgcaa ctcgaccccg 1320
    tgaagtegga gtegetagta ategeagate ageaacgetg eggtgaatac gtteeeggge 1380
    cttgtacaca ccgcccgtca cgtcatgaaa gtcggtaaca cccgaagcca gtggcctaac 1440
    cctcgggagg gagctgtcga aggtgggatc ggcgattggg acgaagtcgt aacaaggtag 1500
    ccgtaccgga aggtgcggct ggatcacctc ctttct
45
    <210> 35
     <211> 1534
     <212> DNA
     <213> Klebsiella pneumoniae
50
    <220>
    <221> modified_base
    <222> (11)..(12)
    <223> N = A, C, G or T/U
55
    <400> 35
    agagtttgat nntggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc 60
```

```
ggtagcacag agagcttgct ctcgggtgac gagcggcgga cgggtgagta atgtctggga 120
     aactgootga tggaggggga taactactgg aaacggtago taataccgca taacgtogca 180
     agaccaaagt gggggacctt cgggcctcat gccatcagat gtgcccagat gggattagct 240
     agtaggtggg gtaacggctc acctaggcga cgatccctag ctggtctgag aggatgacca 300
    gccacactgg aactgagaca cggtccagac tcctacggga ggcagcagtg gggaatattg 360
     cacaatgggc gcaagcctga tgcagccatg ccgcgtgtgt gaagaaggcc ttcgggttgt 420
    aaagcacttt cagcggggag gaaggcgatg aggttaataa cctcatcgat tgacgttacc 480
    ctgcagaaga agcaccggct aactccgtgc cagcagccgc ggtaatacgg agggtgcaag 540
    cgttaatcgg aattactggg cgtaaagcgc acgcaggcgg tctgtcaagt cggatgtgaa 600
10
    atccccgggc tcaacctggg aactgcattc gaaactggca ggctagagtc ttgtagaggg 660
    gggtagaatt ccaggtgtag cggtgaaatg cgtagagatc tggaggaata ccggtggcga 720
    aggcggcccc ctggacaaag actgacgctc aggtgcgaaa gcgtggggag caaacaggat 780
     tagataccct ggtagtccac gccgtaaacg atgtcgattt ggaggttgtg cccttgaggc 840
    gtggcttccg gagctaacgc gttaaatcga ccgcctgggg agtacggccg caaggttaaa 900
15
    actcaaatga attgacgggg gcccgcacaa gcggtggagc atgtggttta attcgatgca 960
     acgcgaagaa ccttacctgg tcttgacatc cacagaactt tccagagatg gattggtgcc 1020
    ttcgggaact gtgagacagg tgctgcatgg ctgtcgtcag ctcgtgttgt gaaatgttgg 1080
    qttaaqtccc qcaacqagcg caacccttat cctttgttgc cagcggttag gccgggaact 1140
    caaaqqaqac tqccaqtqat aaactggagg aaggtgggga tgacqtcaag tcatcatggc 1200
20
    ccttacgacc agggctacac acgtgctaca atggcatata caaagagaag cgacctcgcg 1260
    agagcaagcg gacctcataa agtatgtcgt agtccggatt ggagtctgca actcgactcc 1320
    atgaagtegg aategetagt aategtagat cagaatgeta eggtgaatac gtteeeggge 1380
    cttgtacaca ccgcccgtca caccatggga gtgggttgca aaagaagtag gtagcttaac 1440
    cttcgggagg gcgcttacca ctttgtgatt catgactggg gtgaagtcgt aacaaggtaa 1500
25
    ccgtagggga acctgcggtt ggatcacctc cttt
     <210> 36
     <211> 1485
30
    <212> DNA
     <213> ACTINOBACCILUS ACTIN
    <220>
    <221> modified base
35
     <222> (208)..(1476)
     <223> N = A, C, G or T/U
     <400> 36
    attgaagagt ttgatcatgg ctcagattga acgctggcgg caggcttaac acatgcaagt 60
40
    cggacggtag caggagaaag cttgctttct tgctgacgag tggcggacgg gtgagtaatg 120
    cttgggaatc tgtcttatgg agggggataa cgacgggaaa ctgtcgctaa taccgcgtag 180
    agtcgggaga cgaaagtgcg ggactttntg gccgcatgcc atgagatgag cccaagtgtg 240
    attaggtagt tggtggggta aaggcctacc aagccgacga tcgctagctg gtctgagagg 300
    atggccagcc acaccgggac tgagacacgg cccngactcc tacgggaggc agcagtgggg 360
45
    aatattgege aatgggggea accetgaege agceatgeeg egtgaatgaa gaaggeette 420
    gggttgtaaa gttctttcgg tattgaggaa ggttggtgt ttaatagcat gccaaattga 480
    cgttaaatac aqaaqaagca ccggctaact ccgtgccagc agccgcggta atacgggggg 540
    tgcgagcgtt aatcggaata actgggcgta aagggcacgt aggcggacct ttaagtgagg 600
    tgtgaaatcc ccgggcttaa cctgggnatt gcatttcata ctgggggtct ggagtacttt 660
50
    ngggagggnt agaattccac gtgtagcggt gaaatgcgta gagatgtgga ggaataccga 720
    aggegaagge agcccttgg ggatgtactg acgctgatgt gcgaaagcgt ggggagcaaa 780
    caggattaga taccetggta gtccacgetg taaacggtgt cgatttgggg attggggttt 840
    agccctggtg cccgaagcta acgtgataaa tcgaccgcct ggggagtacg gccgcaaggt 900
    taaaactcaa atgaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960
55
    tgcaacgcga agaaccttac ctactcttga catccgaaga agaactcaga gatgggtttg 1020
    tgccttaggg agctttgaga caggtgctgc atggcngtcg tcagctcgtg ttgtgaaatg 1080
```

WO 03/054162 PCT/US02/41014

```
ttgggttaag tcccgcaacg agcgcaaccc ttatcctttg tggccagcga cgtggtcggg 1140
    aactcaaagg agactgccgg tgataaaccg gaggaaggtg gggatgacgt caagtcatca 1200
    tggcccttac gagtagggct acacacgtgc tacaatggcg tatacagagg gtaaccaacc 1260
    agggatgggg agtgaatctc agaaagtgcg tctaagttcg gattggagtc tgcaactcga 1320
    ctccatgaag toggaatogc tagtaatogc gaatcagaat gttgcggtga atacgttccc 1380
    gggccttgta cacaccgccc gtcacaccat gggagtgggt tgtaccagaa gtggatagct 1440
    gaaccgagag ggtggcgttt accacggtat gattcangac tgggg
10
    <210> 37
    <211> 1487
    <212> DNA
    <213> Haemophilus influenzae
15
    <220>
    <221> modified base
    <222> (1) .. (1387)
    <223> N = A, C, G or T/U
20
    <400> 37
    naattgaaga gtttgatcat ggctcagatt gaacgctggc ggcaggctta acacatgcaa 60
    gtcgaacggt agcaggagaa agcttgcttt cttgctgacg agtggcggac gggtgagtaa 120
    tgcttgggaa tctggcttat ggaggggat aacgacggga aactgtcgct aataccgcgt 180
    attatcggaa gatgaaagtg cgggactgag aggccgcatg ccataggatg agcccaagtg 240
25
    ggattaggta gttggtgggg taaatgccta ccaagcctgc gatctctagc tggtctgaga 300
    ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag gcagcagtgg 360
    ggaatattgc gcnatggggg gaaccctgac gcagccatgc cgcgtgaatg aagaaggcct 420
    tegggttgta aagttettte ggtattgagg aaggttgatg tgttaatage acateaaatt 480
    gacgttaaat acagaagaag caccggctaa ctccgtgcca gcagccgcgg taatacggag 540
30
    ngtgcgagcg ttaatcggaa taactgggcg taaagggcac gcaggcggtt atttaagtga 600
    ggtgtgaaag ccccgggctt aacctgggna ttgcatttca gactgggtaa ctagagtact 660
    ttagggaggg gtagaattcc acgtgtagcg gtgaaatgcg tagagatgtg gaggaatacc 720
    gaaggcgaag gcagccctt gggaatgtac tgacgctcat gtgcgaaagc gtggggagca 780
    aacaggatta gataccetgg tagtecacge tgtaaacget gtegatttgg gggttggggt 840
35
    ttaactctgg cacccgtagc taacgtgata aatcgaccgc ctggggagta cggccgcaag 900
    gttaaaactc aaatgaattg acgggggccn gcacaagcgg tggagcatgt ggtttaattc 960
    gatgcaacgc gaagaacctt acctactctt gacatcctaa gaagagctca gagatgagct 1020
    tgtgccttcg ggaacttaga gacaggtgct gcatggctgt cgtcagctcg tgttgtgaaa 1080
    tgttgggtta agtcccgcaa cgagcgcaac ccttatcctt tgttgccagc gacttggtcg 1140
40
    ggaactcaaa ggagactgcc agtgataaac tggaggaagg tngggatgac gtcaagtcat 1200
    catggccctt acgagtaggg ctacacacgt gctacaatgg cgtatacaga gggaagcgaa 1260
    gctgcgaggt ggagcgaatc tcataaagta cgtctaagtc cggattggag tctgcaactc 1320
    gactecatga agteggaate getagtaate gegaateaga atgtegeggt gaatacgtte 1380
    cogggenttg tacacaccgc cogtcacacc atgggagtgg gttgtaccag aagtagatag 1440
45
    cttaaccttt tggagggcgt ttaccacggt atgattcatg actgggg
                                                                       1487
    <210> 38
    <211> 1532
50
    <212> DNA
    <213> Bordetella bronchiseptica
    <400> 38
    tgaactgaag agtttgatcc tggctcagat tgaacgctgg cgggatgctt tacacatgca 60
55
    agtcggacgg cagcacgggc ttcggcctgg tggcgagtgg cgaacgggtg agtaatgtat 120
    cggaacgtgc ccagtagcgg gggataacta cgcgaaagcg tggctaatac cgcatacgcc 180
```

```
ctacggggga aagcggggga ccttcgggcc tcgcactatt ggagcggccg atatcggatt 240
     agctagttgg tggggtaacg gcctaccaag gcgacgatcc gtagctggtt tgagaggacg 300
     accagecaca etgggaetga gaeaeggeee agaeteetae gggaggeage agtggggaat 360
     tttggacaat gggggcaacc ctgatccagc catcccgcgt gtgcgatgaa ggccttcggg 420
     ttgtaaagca cttttggcag gaaagaaacg gcacgggcta atatcctgtg caactgacgg 480
     tacctgcaga ataagcaccg gctaactacg tgccagcagc cgcggtaata cgtagggtgc 540
     aagcgttaat cggaattact gggcgtaaag cgtgcgcagg cggttcggaa agaaagatgt 600
     gaaatcccag ggcttaacct tggaactgca tttttaacta ccgggctaga gtgtgtcaga 660
     gggaggtgga attccgcgtg tagcagtgaa atgcgtagat atgcggagga acaccgatgg 720
10
     cgaaggcagc ctcctgggat aacactgacg ctcatgcacg aaagcgtggg gagcaaacag 780
     gattagatac cctggtagtc cacgccctaa acgatgtcaa ctagctgttg gggccttcgg 840
     gccttggtag cgcagctaac gcgtgaagtt gaccgcctgg ggagtacggt cgcaagatta 900
     aaactcaaag gaattgacgg ggacccgcac aagcggtgga tgatgtggat taattcgatg 960
     caacgegaaa aaccttacct acccttgaca tgtctggaat cccgaagaga tttgggagtg 1020
15
     ctcgcaagag aaccggaaca caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg 1080
     ttgggttaag tcccgcaacg agcgcaaccc ttgtcattag ttgctacgaa agggcactct 1140
     aatgagactg ccggtgacaa accggaggaa ggtggggatg acgtcaagtc ctcatggccc 1200
     ttatgggtag ggcttcacac gtcatacaat ggtcgggaca gagggtcgcc aacccgcgag 1260
     ggggagccaa tcccagaaac ccgatcgtag tccggatcgc agtctgcaac tcgactgcgt 1320
20
     gaagteggaa tegetagtaa tegeggatea geatgtegeg gtgaataegt teeegggtet 1380
     tgtacacacc gcccgtcaca ccatgggagt gggttttacc agaagtagtt agcctaaccg 1440
     caagggggc gattaccacg gtaggattca tgactggggt gaagtcgtaa caaggtagcc 1500
     gtatcggaag gtgcggctgg atcacctcct tt
                                                                       1532
25
     <210> 39
     <211> 1485
     <212> DNA
     <213> Bordetella parapertussis
30
     <400> 39
     attgaacget ggcgggatge tttacacatg caagtcggac ggcagcacgg getteggeet 60
     ggtggcgagt ggcgaacggg tgagtaatgt atcggaacgt gcccagtagc gggggataac 120
     tacgcgaaag cgtggctaat accgcatacg ccctacgggg gaaagcgggg gactttcggg 180
35
     cctcgcacta ttggagcggc cgatatcgga ttagctagtt ggtggggtaa cggcctacca 240
     aggcgacgat ccgtagctgg tttgagagga cgaccagcca cactgggact gagacacggc 300
     ccagactcct acgggaggca gcagtgggga attttggaca atgggggcaa ccctgatcca 360
     gccatcccgc gtgtgcgatg aaggccttcg ggttgtaaag cacttttggc aggaaagaaa 420
     cggcacgggc taatatcctg tgcaactgac ggtacctgca gaataagcac cggctaacta 480
40
     cgtgccagca gccgcggtaa tacgtagggt gcaagcgtta atcggaatta ctgggcgtaa 540
     agcgtgcgca ggcggttcgg aaagaaagat gtgaaatccc agggcttaac cttggaactg 600
     catttttaac taccgggcta gagtgtgtca gagggaggtg gaattccgcg tgtagcagtg 660
     aaatgcgtag atatgcggag gaacaccgat ggcgaaggca gcctcctggg ataacactga 720
     cgctcatgca cgaaagcgtg gggagcaaac aggattagat accctggtag tccacgccct 780
     aaacgatgtc aactagctgt tggggccttc gggccttggt agcgcagcta acgcgtgaag 840
     ttgaccgcct ggggagtacg gtcgcaagat taaaactcaa aggaattgac ggggacccgc 900
     acaagcggtg gatgatgtgg attaattcga tgcaacgcga aaaaccttac ctacccttga 960
     catgtctgga atcccgaaga gatttgggag tgctcgcaag agaaccggaa cacaggtgct 1020
    gcatggctgt cgtcagctcg tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac 1080
50
    ccttgtcatt agttgctacg aaagggcact ctaatgagac tgccggttac aaaccggagg 1140
     aaggtgggga tgacgtcaag tcctcatggc ccttatgggt agggcttcac acgtcataca 1200
    atggteggga cagagggteg ccaaccegeg agggggagec aatcecagaa accegategt 1260
    agtccggatc gcagtctgca actcgactgc gtgaagtcgg aatcgctagt aatcgcggat 1320
    cagcatgtcg cggtgaatac gttcccgggt cttgtacaca ccgcccgtca caccatggga 1380
55
    gtgggtttta ccagaagtag ttagcctaac cgcaaggggg gggcgattac cacggtagga 1440
```

ttcatgactg gggtgaagtc gtaacaaggt agccgtatcg gaagg

WO 03/054162 PCT/US02/41014

```
<210> 40
     <211> 1464
     <212> DNA
     <213> Bordetella pertussis
     <220>
     <221> modified base
10
     <222> (87) .. (1391)
     <223> N = A, C, G or T/U
     <400> 40
     aactgaagag tttgatcctg gctcagattg aacgctggcg ggatgcttta cacatgcaag 60
15
     teggacggca geacgggett eggeetngtg gegagtggeg aaegggtgag taatgtateg 120
     gaacgtgccc agtagcgggg gataactacg cgaaagcgta gctaataccg catacgccct 180
     acgggggaaa gcgggggacc ttcgggcctc gcactattgg agcggccgat atcggattag 240
     ctngttggtg gggtaacggc ctaccaaggc gacgatccgt agctggtttg agaggacgac 300
     cagccacact gggactgaga cacggcccag netectacgg gaggcagcag tggggaattt 360
20
     tggacaatgg gggcaaccct gatccagcca tcccgcgtgt gcgatgaagg ccttcgggtt 420
     gtaaagcact tttggcagga aagaaacggc acgggctaat atcctgtgca actgacggta 480
     cctgcagaat aagcaccggc taactacgtg ccagcagccg cggtaatacg tagggtgcaa 540
     gcgttaatcg gaattactgg gcgtaaagcg tgcgcaggcg gttcggaaag aaagatgtga 600
     aatcccaggg cttaaccttg gaactgcatt tttaactacc gggctagagt gtgtcagagg 660
25
     gaggtggaat teegegtgta geagtgaaat gegtagatat geggaggaae acegatggeg 720
     aaggcagcct cctgggataa cactgacgct catgcacgaa agtgtgggga gcaaacagga 780
     ttagatacce tggtagteca cgccctaaac gatgtcaact agctgttggg gccttcgggc 840
    cttggtagcg cagctaacgc gtgaagttga ccgcctgggg agtacggtcg caagattaaa 900
     actcaaagga attgacgggg acccgcacaa gcggtggatg atgtggatta attcgatgca 960
    acgcgaaaaa ccttacctac ccttgacatg tctggaatcc cgaagagatt tgggagtgct 1020
     cgcaagagaa ccggaacaca ggtgctgcat ggctgtcgtc agctcgtgtc gtgagatgtt 1080
     gggttaagtc ccgcaacgag cgcaaccctt gtcattagtt gctacgaaag ggcactctaa 1140
     tgagactgcc ggtgacaaac cggaggaagg tggggatgac gtgaagtcct catggccctt 1200
     atgggtaggg cttcacacgt catacaatgg tegggacaga gggttgncaa cccgegaggg 1260
35
     ggagccaatc ccagaaaccc ggtcgtngtc cggatcgcag tctgcaactc gactgcgtga 1320
     agtoggaato gotagtaato goggatoago atgtogoggt gaatacgtto cogggtottg 1380
     tacacaccgc ncgtcacacc atgggagtgg gttttaccag aagtagttag cctaaccgca 1440
     agggggggga ttaccacggt agga
40
    <210> 41
     <211> 1535
     <212> DNA
     <213> Burkholderia cepacia
45
     <400> 41
     taaactgaag agtttgatcc tggctcagat tgaacgctgg cggcatgctt aacacatgca 60
    agtcgaacgg cagcacgggt gcttgcacct ggtggcgagt ggcgaacggg tgagtaatac 120
    atoggaacat gtootgtagt gggggatago coggcgaaag coggattaat accgcatacg 180
50
    atctacggat gaaagcgggg gaccttcggg cctcgcgcta tagggttggc gatggctgat 240
    tagctagttg gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac 300
    gaccagecae actgggaetg agacaeggee cagaeteeta egggaggeag cagtggggaa 360
    ttttggacaa tgggcgaaag cctgatccag caatgccgcg tgtgtgaaga aggccttcgg 420
    gttgtaaagc acttttgtcc ggaaagaaat ccctggctct aatacagtcg ggggatgacg 480
55
    gtaccggaag aataagcacc ggctaactac gtgccagcag ccgcggtaat acgtagggtg 540
    caagegttaa teggaattae tgggegtaaa gegtgegeag geggtttget aagacegatg 600
```

```
tgaaatcccc gggctcaacc tgggaactgc attggtgact ggcaggctag agtatggcag 660
     aggggggtag aattccacgt gtagcagtga aatgcgtaga gatgtggagg aataccgatg 720
     gcgaaggcag ccccctgggc caatactgac gctcatgcac gaaagcgtgg ggagcaaaca 780
     qqattaqata ccctggtagt ccacgcccta aacgatgtca actagttgtt ggggattcat 840
     ttccttagta acgtagctaa cgcgtgaagt tgaccgcctg gggagtacgg tcgcaagatt 900
     aaaactcaaa ggaattgacg gggacccgca caagcggtgg atgatgtgga ttaattcgat 960
     gcaacgcgaa aaaccttacc tacccttgac atggtcggaa tcctgctgag aggtgggagt 1020
     gctcgaaaga gaaccggcgc acaggtgctg catggctgtc gtcagctcgt gtcgtgagat 1080
     gttgggttaa gtcccgcaac gagcgcaacc cttgtcctta gttgctacgc aagagcactc 1140
10
     taaggagact gccggtgaca aaccggagga aggtggggat gacgtcaagt cctcatggcc 1200
     cttatgggta gggcttcaca cgtcatacaa tggtcggaac agagggttgc caacccgcga 1260
     gggggagcta atcccagaaa acccatcgta gtccggattg cactctgcaa ctcgagtgca 1320
     tgaagetgga ategetagta ategeggate ageatgeege ggtgaataeg tteeegggte 1380
     ttgtacacac cgcccgtcac accatgggag tgggttttac cagaagtggc tagtctaacc 1440
15
     gcaaggagga cggtcaccac ggtaggattc atgactgggg tgaagtcgta acaaggtagc 1500
     cgtatcggaa ggtgcggctg gatcacctcc tttct
     <210> 42
20
     <211> 1488
     <212> DNA
     <213> Burkholderia mallei
     <400> 42
25
     agattgaacg ctggcggcat gccttacaca tgcaagtcga acggcagcac gggcttcggc 60
     ctggtggcga gtggtgaacg ggtgagtaat acatcggaac atgtcctgta gtgggggata 120
     gcccggcgaa agccggatta ataccgcata cgatctgagg atgaaagcgg gggaccttcg 180
     ggcctcgcgc tatagggttg gccgatggct gattagctag ttggtggggt aaaggcctac 240
     caaggegacg atcagtaget ggtetgagag gacgaccage cacactggga etgagacacg 300
30
     gcccagactc ctacgggagg cagcagtggg gaattttgga caatgggcgc aagcctgatc 360
     cagcaatgcc gcgtgtgtga agaaggcctt cgggttgtaa agcacttttg tccggaaaga 420
     aatcattctg gctaataccc ggagtggatg acggtaccgg aagaataagc accggctaac 480
     tacgtgccag cagccgcggt aatacgtagg gtgcgagcgt taattggaat tactgggcgt 540
     aaagcgtgcg caggcggttt gctaagaccg atgtgaaatc cccgggctca acctgggaac 600
35
     tgcattggtg actggcaggc tagagtatgg cagagggggg tagaattcca cgtgtagcag 660
     tgaaatgcgt agagatgtgg aggaataccg atggcgaagg cagccccctg ggccaatact 720
    gacgctcatg cacgaaagcg tggggagcaa acaggattag ataccctggt agtccacgcc 780
     ctaaacgatg tcaactagtt gttggggatt catttcctta gtaacgtagc taacgcgtga 840
     agttgaccgc ctggggagta cggtcgcaag attaaaactc aaaggaattg acggggaccc 900
40
    gcacaagcgg tggatgatgt ggattaattc gatgcaacgc gaaaaacctt acctaccctt 960
    gacatggtcg gaagcccgat gagagttggg cgtgctcgaa agagaaccgg cgcacaggtg 1020
     ctgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca 1080
     accettgtcc ttagttgcta cgcaagagca ctctaaggag actgccggtg acaaaccgga 1140
    ggaaggtggg gatgacgtca agtcctcatg gcccttatgg gtagggcttc acacgtcata 1200
45
     caatggtcgg aacagagggt cgccaacccg cgagggggag ccaatcccag aaaaccgatc 1260
    gtagtccgga ttgcactctg caactcgagt gcatgaagct ggaatcgcta gtaatcgcgg 1320
     atcagcatgc cgcggtgaat acgttcccgg gtcttgtaca caccgcccgt cacaccatgg 1380
     gagtgggttt taccagaagt ggctagtcta accgcaagga ggacggtcac cacggtagga 1440
     ttcatgactg gggtgaagtc gtaacaaggt agccgtatcg gaaggtgc
50
     <210> 43
     <211> 1610
     <212> DNA
55
     <213> Burkholderia pseudomallei
```

```
<400> 43
     tctagatgcg tgctcgagcg gccgcccagt gctgcatgga tatctgctga attcggcttg 60
     agcagtttga teetggetea gattgaaege tggeggeatg cettacacat gcaagtegaa 120
     cggcagcacg ggcttcggcc tggtggcgag tggcgaacgg gtgagttata catcggagca 180
    tgtcctgtag tgggggatag cccggcgaaa gccgaattaa taccgcatac gatctgagga 240
     tgaaagcggg ggaccttcgg gcctcgcgct atagggttgg ccgatggctg attagctagt 300
    tggtggggta aaggectacc aaggegacga teagtagetg gtetgagagg acgaccagec 360
    acactgggac tgagacacgg cccagactcc tacgggaggc agcagtgggg aattttggac 420
    aatgggcgca agcctgatcc agcaatgccg cgtgtgtgaa gaaggccttc gggttgtaaa 480
    gcacttttgt ccggaaagaa atcattctgg ctaatacccg gagtggatga cggtaccgga 540
10
    aqaataagca ccggctaact acgtgccagc agccgcggta atacgtaggg tgcgagcgtt 600
    aatcgggatt actgggcgta aagcgtgcgc aggcggtttg ctaagaccga tgtgaaatcc 660
    ccgggctcaa cctgggaact gcattggtga ctggcaggct agagtatggc agaggggggt 720
    agaattccac gtgtagcagt gaaatgcgta gagatgtgga ggaataccga tggcgaaggc 780
15
    ageceeetgg gecaatactg acgeteatge acgaaagegt ggggagaaaa caggattaga 840
    taccetggta gtccacgccc taaacgatgt caactagttg ttggggattc atttccttag 900
    taacgtagct aacgcgcgaa gttgaccgcc tggggagtac ggtcgcaaga ttaaaactca 960
    aaggaattga cggggacccg cacaagcggt ggatgatgtg gattaattcg atgcaacgcg 1020
    aaaaacctta cctacccttg acatggtcgg aagcccgatg agagttgggc gtgctcgaaa 1080
20
    gagaaccggc gcacaggtgc tgcatggctg tcgtcagctc gtgtcgtgag atgttgggtt 1140
    aagtcccgca acgagcgcaa cccttgtcct tagttgctac gcaagagcac tctaaggaga 1200
    ctgccggtga caaaccggag gaaggtgggg atgacgtcaa gtcctcatgg cccttatggg 1260
     tagggettea caegteatae aatggtegga acagagggte gecaaceege gagggggage 1320
    caatcccaga aaaccgatcg tagtccggat tgcactctgc aactcgagtg catgaagctg 1380
25
    gaategetag taategegga teageatgee geggtgaata egtteeeggg tettgtacae 1440
    accgcccgtc acaccatggg agtgggtttt accagaagtg gctagtctaa ccgcaaggag 1500
    gacggtcacc acggtaggat tcatgactgg ggtgaagtcg taacaaggta gccgtagaag 1560
    ccgaattcca gcacactggc ggccgttact actggatccg agctcgtacc
30
    <210> 44
     <211> 1544
     <212> DNA
     <213> Neisseria gonorrhoeae
35
     <400> 44
     tgaacataag agtttgatcc tggctcagat tgaacgctgg cggcatgctt tacacatgca 60
     agtcggacgg cagcacaggg aagcttgctt ctcgggtggc gagtggcgaa cgggtgagta 120
    acatategga acgtaceggg tageggggga taactgateg aaagateage taatacegca 180
40
    tacgtcttga gagggaaagc aggggacctt cgggccttgc gctatccgag cggccgatat 240
     ctgattagct ggttggcggg gtaaaggccc accaaggcga cgatcagtag cgggtctgag 300
     aggatgatcc gccacactgg gactgagaca cggcccagac tcctacggga ggcagcagtg 360
     gggaattttg gacaatgggc gcaagcctga tccagccatg ccgcgtgtct gaagaaggcc 420
     ttcgggttgt aaaggacttt tgtcagggaa gaaaaggctg ttgccaatat cggcggccga 480
45
     tgacggtacc tgaagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta 540
    gggtgcgagc gttaatcgga attactgggc gtaaagcggg cgcagacggt tacttaagca 600
     ggatgtgaaa teeceggget caaceeggga aetgegttet gaactgggtg aetegagtgt 660
     gtcagaggga ggtggaattc cacgtgtagc agtgaaatgc gtagagatgt ggaggaatac 720
     cgatggcgaa ggcagcctcc tgggataaca ctgacgttca tgtccgaaag cgtgggtagc 780
50
    aaacaggatt agataccctg gtagtccacg ccctaaacga tgtcaattag ctgttgggca 840
     acttgattgc ttggtagcgt agctaacgcg tgaaattgac cgcctgggga gtacggtcgc 900
     aagattaaaa ctcaaaggaa ttgacgggga cccgcacaag cggtggatga tgtggattaa 960
     ttcgatgcaa cgcgaagaac cttacctggt tttgacatgt gcggaatcct ccggagacgg 1020
     55
     agatgttggg ttaagtcccg caacgagcgc aaccettgtc attagttgcc atcattcggt 1140
     tgggcactet aatgagactg ceggtgacaa geeggaggaa ggtggggatg aegteaagte 1200
```

```
ctcatggccc ttatgaccag ggcttcacac gtcatacaat ggtcggtaca gagggtagcc 1260
     aageegegag geggageeaa teteacaaaa eegategtag teeggattge actetgeaac 1320
     tegagtgeat gaagteggaa tegetagtaa tegeaggtea geatactgeg gtgaataegt 1380
     tcccgggtct tgtacacacc gcccgtcaca ccatgggagt ggggggatacc agaagtaggt 1440
     agggtaaccg caaggagtcc gcttaccacg gtatgcttca tgactggggt gaagtcgtaa 1500
     caaggtagcc gtaggggaac ctgcggctgg atcacctcct ttct
     <210> 45
10
     <211> 1544
     <212> DNA
     <213> Neisseria meningitidis
     <400> 45
15
     tgaacataag agtttgatcc tggctcagat tgaacgctgg cggcatgctt tacacatgca 60
     agteggaegg cageacagag aagettgett etegggtgge gagtggegaa egggtgagta 120
     acatatogga acgtacogag tagtggggga taactgatog aaagatoago taatacogca 180
     tacgtcttga gagagaaagc aggggacctt cgggccttgc gctattcgag cggccgatat 240
     ctgattagct agttggtggg gtaaaggcct accaaggcga cgatcagtag cgggtctgag 300
20
     aggatgatee gecacaetgg gaetgagaea eggeeeagae teetaeggga ggeageagtg 360
     gggaattttg gacaatgggc gcaagcctga tccagccatg ccgcgtgtct gaagaaggcc 420
     ttcgggttgt aaaggacttt tgtcagggaa gaaaaggctg ttgctaatat cagcggctga 480
     tgacggtacc tgaagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta 540
     gggtgcgagc gttaatcgga attactgggc gtaaagcggg cgcagacggt tacttaagca 600
25
     ggatgtgaaa tccccgggct caacccggga actgcgttct gaactgggtg actcgagtgt 660
     gtcagaggga ggtagaattc cacgtgtagc agtgaaatgc gtagagatgt ggaggaatac 720
     cgatggcgaa ggcagcctcc tgggacaaca ctgacgttca tgcccgaaag cgtgggtagc 780
     aaacaggatt agataccctg gtagtccacg ccctaaacga tgtcaattag ctgttgggca 840
     acctgattgc ttggtagcgt agctaacgcg tgaaattgac cgcctgggga gtacggtcgc 900
30
     aagattaaaa ctcaaaggaa ttgacgggga cccgcacaag cggtggatga tgtggattaa 960
     ttcgatgcaa cgcgaagaac cttacctggt cttgacatgt acggaatcct ccggagacgg 1020
     aggagtgcct tegggageeg taacacaggt getgeatgge tgtegteage tegtgtegtg 1080
     agatgttggg ttaagtcccg caacgagegc aaccettgte attagttgce atcattcagt 1140
     tgggcactct aatgagactg ccggtgacaa gccggaggaa ggtggggatg acgtcaagtc 1200
35
     ctcatggccc ttatgaccag ggcttcacac gtcatacaat ggtcggtaca gagggtagcc 1260
     aagccgcgag gcggagccaa tctcacaaaa ccgatcgtag tccggattgc actctgcaac 1320
     tcgagtgcat gaagtcggaa tcgctagtaa tcgcaggtca gcatactgcg gtgaatacgt 1380
     tecegggtet tgtacacace gecegteaca ceatgggagt gggggatace agaagtaggt 1440
     aggataacca caaggagtcc gcttaccacg gtatgcttca tgactggggt gaagtcgtaa 1500
40
     caaggtagcc gtaggggaac ctgcggctgg atcacctcct ttct
     <210> 46
     <211> 1537
45
     <212> DNA
     <213> Pseudomonas aeruginosa
     <400> 46
     gaactgaaga gtttgatcat ggctcagatt gaacgctggc agcaggggcc ttcaacacat 60
50
     gcaagtcgag cttatgaagg gagcttgcct tggattcagc ggcggacggg tgagtaatgc 120
     ctaggaatct gcctggtagt gggggataac gtccggaaac ggccgctaat accgcatacg 180
     tcctgaggga gaaagtcggg gatcttcgga cctcacgcta tcagatgagc ctaggtcgga 240
     ttagctagtt ggtggggtaa aggcctacca aggcgacgat ccgtaactgg tctgagagga 300
     tqatcaqtca cactqqaact gagacacggt ccaqactcct acqqqaqqca gcagtqqqqa 360
55
     atattggaca atgggcgcaa gcctgatcca gccatgccgc gtgtgtgaag aaggtcttcg 420
     gattgtaaag cactttaagt tgggaggaag ggcagtaagt taataccttg ctgtttgacg 480
```

```
ttaccaacag aataagcacc ggctaacttc gtgccagcag ccgcggtaat acgaagggtg 540
     caagcgttaa tcggaattac tgggcgtaaa gcgcgcgtaa gtggttcagc aagcttgatg 600
     tgaaatcccc gggctcaacc tgggaactgc atccaaaagc tactgagcta gagtacggta 660
     gaggtggtag aatttcctgt gtagcggtga aatgcgtaga tataggaagg aacaccagtg 720
    gcgaaggcga ccacctggac tgtactgaca ctgaggtgcg aaagcgtggg gagcaaacag 780
    gattagatac cetggtagte caegeegtaa aegatgtega etageegttg ggateettga 840
    gatettagtg gegeacgtaa egegataagt egacegeetg gggagtaegg eegeaaggtt 900
    aaaactcaaa tgaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcgaa 960
    gcaacgcgaa gaaccttacc tggccttgac atgctgagaa ctttccagag atggattggt 1020
10
    gccttcggga acagagacac aggtgctgca tggctgtcgt cagctcgtgt cgtgagatgt 1080
     tgggttaagt cccgtaacga gcgcaaccct tgtccttagt taccagcacc tcgggtgggc 1140
     actctaagga gactgccggt gacaaaccgg aggaaggtgg ggatgacgtc aagtcatcat 1200
    ggcccttacg gccagggcta cacacgtgct acaatggtcg gtacaaaggg ttgccaagcc 1260
    gcgagtggga gctaatccca taaaaccgat cgtagtccgg atcgcagtct gcaactcgac 1320
15
     tgcgtgaagt cggaatcgct agtaatcgtg aatcagaatg tcacggtgaa tacgtccccg 1380
     ggccttgtac acaccgcccg tcacaccatg ggagtgggtt gctccagaag tagctagtct 1440
     aaccgcaagg gggacggtta ccacggagtg attcatgact ggggtgaagt cgtaacaagg 1500
     tagecgtagg ggaacetgeg getggateae eteetta
20
     <210> 47
     <211> 1467
     <212> DNA
     <213> Vibrio cholerae
25
     <220>
     <221> modified base
     <222> (928)..(1464)
     <223> N = A, C, G or T/U
30
     <400> 47
     attgaagagt ttgatcctgg ctcagattga acgctggcgg caggcctaac acatgcaagt 60
     cgagcggcag cacagaggaa cttgttcctt gggtggcgag cggcggacgg gtgagtaatg 120
     cctgggaaat tgcccggtag agggggataa ccattggaaa cgatggctaa taccgcataa 180
35
     cctcgcaaga gcaaagcagg ggaccttcgg gccttgcgct accggatatg cccaggtggg 240
     attagctagt tggtgaggta agggctcacc aaggcgacga tccctagctg gtctgagagg 300
     atgateagee acactggaae tgagacaegg tecagaetee taegggagge ageagtgggg 360
     aatattgcac aatgggcgca agcctgatgc agccatgccg cgtgtatgaa gaaggccttc 420
    gggttgtaaa gtactttcag tagggaggaa ggtggttaag ttaatacctt aatcatttga 480
40
     cgttacctac agaagaagca ccggctaact ccgtgccagc agccgcggta atacggaggg 540
     tgcaagcgtt aatcggaatt actgggcgta aagcgcatgc aggtggtttg ttaagtcaga 600
     tgtgaaagcc ctgggctcaa cctaggaatc gcatttgaaa ctgacaagct agagtactgt 660
     agagggggt agaatttcag gtgtagcggt gaaatgcgta gagatctgaa ggaataccgg 720
     tggcgaaggc ggcccctgg acagatactg acactcagat gcgaaagcgt ggggagcaaa 780
45
     caggattaga taccctggta gtccacgccg taaacgatgt ctacttggag gttgtgccct 840
     agagtegtgg ettteggage taaegegtta agtagaeege etggggagta eggtegeaag 900
     attaaaactc aaatqaattg acgggggncc gcacaagcgg tggagcatgt ggtttaattc 960
     ganncaacgc gaagaacctt acctactctt gacatccaga gaatctagcg gagacgctgg 1020
     agtgccttcg ggagctctga gacaggtgct gcatggctgt cgtcagctcg tgttgtgaaa 1080
50
     tgttgggtta agtcccgcaa cgagcgcaac ccttatcctt gtttgccagc acgtaatggt 1140
    gggaactcca gggagactgc cggtgataaa ccggaggaag gtggggacga cgtcaagtca 1200
     tcatggccct tacgagtagg gctacacacg tgctacaatg gcgtatacag agggcagcga 1260
     taccgcgagg tggagcgaat ctcacaaagt acgtcgtagt ccggattgga gtctgcaact 1320
     cgactccatg aagtcggaat cgctagtaat cgcaaatcag aatgttgcgg tgaatacgtt 1380
55
     cccgggcctt gtacacaceg cccgtcacac catgggagtg ggctgcaaaa gaagcangta 1440
```

gtttaacctt cgggaggacg cttnccc

WO 03/054162 PCT/US02/41014

```
<210> 48
    <211> 1485
    <212> DNA
    <213> Yersinia enterocolitica
    <220>
    <221> modified base
10
    <222> (1)..(1484)
     <223> N = A, C, G or T/U
    <400> 48
    naattgaaga gtttgatcat ggctcagatn gaacgctggc ggcaggccta acacatgcaa 60
15
    gtcgagcggc agcgggaagn agtttactac tttcngggcg agcggcgnac gggtgagtaa 120
    tgtctgggaa actgcctgat ggagggggat aactactgga aacggtagct aataccgcat 180
    aacgtetteg gaccaaagtg ggggacetta gggceteaeg ceatengatg tgeecagatg 240
    ggattagcta gtaggtgggg taatggctca cctaggcgac gatccctagc tggtctgaga 300
    ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag gcagcagtgg 360
20
    ggaatattgc acaatgggcg caagcctgat gcagccatgc cgcgtgtgtg aagaaggcct 420
    tcgggttgta aagcactttc agcgaggagg aaggccaata acttaatacg ttgttggatt 480
    qacqttactc qcaqaaqaaq caccqqctaa ctccgtgcca gcagccgcgg taatacggag 540
    ggtgcaagcg ttaatcggaa ttactgggcg taaagcgcac gcaggcggtt tgttaagtca 600
    gatgtgaaat ccccgcgctt aacgtgggna cngcatttga aactggcaag ctagagtctt 660
25
    gtagagggg gtagaattcc aggtgtagcg gtgaaatgcg tagagatctg naggaatacc 720
    ggtggcgaag gcggccccct ggacaaagac tgacgctcag gtgcgaaagc gtggggagca 780
    aacaggatta gataccctgg tagtccacgc tgtaaacgat gtcgacttgg aggttgtgcc 840
    cttgaggegt ggetteegga getaaegegt taagtegace geetggggag taeggeegea 900
    aggttaaaac tcaaatgaat tnncgggggc cngcacaagc ggtggagcat gtggtttaat 960
    tcgatgcaac gcgaagaacc ttacctactc ttgacatcca cggaatttag cagagatgct 1020
    ttagtgnett egggaacegt gagaeaggtg etgeatgget gtegteaget egtgttgtga 1080
    aatgttgggt taagtcccgc aacgagcgca acccttatcc tttgttgcca gcacgtaatg 1140
    gtgggaactc aaaggagact gccggtgata aaccggagga aggtggggat gacgtcaagt 1200
    catcatggcc cttacgagta gggctacaca cgtgctacaa tggcagatac aaagtgaagc 1260
35
    gaactcgcga gagcaagcgg accacataaa gtctgtcgta gtccggattg gagtctgcaa 1320
    ctcgactcca tgaagtcgga atcgctagta atcgtagatc agaatgctac ggtgaatacg 1380
    ttcccgggcc ttgtacacac cgcccgtcac accntgggag tgggttgcaa aagaagtagg 1440
    tagettaaen ttegggaggg egegtaeeae tttgtgatte nngne
40
    <210> 49
    <211> 2927
     <212> DNA
    <213> Bacillus subtilis
45
     <400> 49
    qqttaaqtta qaaaqggcgc acggtggatg ccttggcact aggagccgat gaaggacggg 60
    acgaacaccg atatgetteg gggagetgta ageaagettt gateeggaga ttteegaatg 120
    gggaaaccca ccactcgtaa tggagtggta tccatatctg aattcatagg atatgagaag 180
50
    gcagaccegg ggaactgaaa catctaagta cceggagaag agaaagcaaa tgegatteec 240
     tgagtagegg egaegaacae gggateagee caaaceaaga ggettgeete tgtggttgta 300
    ggacactetg taeggagtta caaaagaaeg aggtagatga agaggtetgg aaagggeeeg 360
    ccataggagg taacagccct gtagtcaaaa cttcgttctc tcctgagtgg atcctgagta 420
    cggcggaaca cgtgaaattc cgtcggaatc cgggaggacc atctcccaag gctaaatact 480
55
    ccctagtgac cgatagtgaa ccagtaccgt gagggaaagg tgaaaagcac cccggaaggg 540
    gagtgaaaga gatcctgaaa ccgtgtgcct acaagtagtc agagcccgtt aacggtgatg 600
```

```
gcgtgccttt tgtagaatga accggcgagt tacgatcccg tgcaaggtta agcagaagat 660
     geggageege agegaaageg agtetgaata gggegeatga gtaegtggte gtagaeeega 720
     aaccaggtga tctacccatg tccagggtga agttcaggta acactgaatg gaggcccgaa 780
     cccacgcacg ttgaaaagtg cggggatgag gtgtgggtag gggtgaaatg ccaatcgaac 840
     ctggagatag ctggttctct ccgaaatagc tttagggcta gcctcaaggt aagagtcttg 900
     gaggtagagc actgattgga ctaggggccc tcaccgggtt accgaattca gtcaaactcc 960
     gaatgccaat gacttatect tgggagtcag actgcgagtg ataagatecg tagtegaaag 1020
     ggaaacagcc cagaccgcca gctaaggtcc caaagtatac gttaagtgga aaaggatgtg 1080
     gagttgctta gacaaccagg atgttggctt agaagcagcc accatttaaa gagtgcgtaa 1140
10
     tageteactg gtegagtgae tetgegeega aaatgtaceg gggetaaacg tateacegaa 1200
     gctgcggact gttcttcgaa cagtggtagg agagcgttct aagggctgtg aagccagacc 1260
    ggaaggactg gtggacggct tagaagtgag aatgccggta tgagtagcga aaagaggggt 1320
    qaqaatccct ccaccgaatg cctaaqggtt cctgaggaag gctcgtccgc tcagggttag 1380
     tegggaeeta ageegaggee gaaaggegta ggegatggae aacaggttga tatteetgta 1440
15
     ccacctcctc accatttgag caatgggggg tcgcaggagg atagggtaag cgcggtattg 1500
    gatatccgcg tccaagcagt taggctggga aataggcaaa tccgtttccc ataaggctga 1560
    gctgtgatgg cgagcgaaat atagtagcga agttcctgat tccacactgc caagaaaagc 1620
    ctctagcgag gtgagaggtg cccgtaccgc aaaccgtcac aggtaggcga ggagagaatc 1680
    ctaaggtgat cgagagaact ctcgttaagg aactcggcaa aatgaccccg taacttcggg 1740
20
     agaaggggtg ctctgttagg gtgcaagccc gagagagccg cagtgaatag gcccaggcga 1800
     ctgtttagca aaaacacagg tctctgcgaa gccgtaaggc gaagtatagg ggctgacgcc 1860
     tgcccggtgc tggaaggtta agaggagcgc ttagcgtaag cgaaggtgcg aattgaagcc 1920
     ccagtaaacg gcggccgtaa ctataacggt cctaaggtag cgaaattcct tgtcgggtaa 1980
    gttccgaccc gcacgaaagg cgcaacgatc tgggcgctgt ctcaacgaga gactcggtga 2040
25
     aattatagta cctgtgaaga tgcaggttac ccgcgacagg acggaaagac cccgtggagc 2100
     tttactgcag cctgatattg aatgttggta cagcttgtac aggataggta ggagccttgg 2160
     aaaccggagc gccagcttcg gtggaggcat cggtgggata ctaccctggc tgtattgacc 2220
     ttctaacccc ccgcccttat cgggcgggga gacagtgtca ggtgggcagt ttgactgggg 2280
     cggtcgcctc ctaaaaggta acggaggcgc ccaaaggttc cctcagaatg gttggaaatc 2340
30
    attcgcagag tgtaaaggca caagggagct tgactgcgag acctacaagt cgagcaggga 2400
    cgaaagtcgg gcttagtgat ccggtggttc cgcatggaag ggccatcgct caacggataa 2460
    aagctacccc ggggataaca ggcttatctc ccccaagagc tccacatcga cggggaggtt 2520
    tggcacctcg atgtcggctc atcgcatcct ggggctgtag tcggtcccaa gggttgggct 2580
    gttcgcccat taaagcggta cgcgagctgg gttcagaacg tcgtgagaca gttcggtccc 2640
35
    tatccgtcgc gggcgctgga aatttgagag gagctgtcct tagtacgaga ggaccgggat 2700
    ggacgcaccg ctggtgtacc agttgttctg ccaagggcat cgctgggtag ctatgtgcgg 2760
     acgggataag tgctgaaagc atctaagcat gaagcccccc tcaagatgag atttcccatt 2820
    ccgcaaggaa gtaagatccc tgaaagatga tcaggttgat aggtctgagg tggaagtgtg 2880
    gcaacacatg gagctgacag atactaatcg atcgaggact taaccat
40
    <210> 50
     <211> 2922
     <212> DNA
45
     <213> Bacillus anthracis
     <400> 50
    ggttaagtta gaaagggcgc acggtggatg ccttgacact aggagtcgat gaaggacggg 60
    actaacgccg atatgcttcg gggagctgta agtaagcttt gatccgaaga tttccgaatg 120
50
    gggaaaccca ccatacgtaa tggtatggta tccttatctg aatacatagg gtaaggaaga 180
    cagacccagg gaactgaaac atctaagtac ctggaggaag agaaagcaaa tgcgatttcc 240
    tgagtagogg cgagcgaaac ggaacatagc ccaaaccaag aggcttgcct cttggggttg 300
    taggacatte tatacggagt tacaaaggaa cgaggtagac gaagcgacet ggaaaggtee 360
    gtcgtagagg gtaacaaccc cgtagtcgaa acttcgttct ctcttgaatg tatcctgagt 420
55
    acggcggaac acgtgaaatt ccgtcggaat ctgggaggac catctcccaa ggctaaatac 480
    tccctagtga tcgatagtga accagtaccg tgagggaaag gtgaaaagca ccccggaagg 540
```

```
ggagtgaaag agatcctgaa accgtgtgcc tacaaatagt cagagcccgt taacgggtga 600
     tggcgtgcct tttgtagaat gaaccggcga gttacgatcc cgtgcgaggt taagctgaag 660
     aggeggagee geagegaaag egagtetgaa tagggegttt agtaegtggt egtagaeeeg 720
     aaaccaggtg atctacccat gtccagggtg aagttcaggt aacactgaat ggaggcccga 780
     acceaegeae gttgaaaagt geggggatga ggtgtgggta geggagaaat tecaategaa 840
     cctggagata gctggttctc cccgaaatag ctttagggct agccttaagt gtaagagtct 900
     tggaggtaga gcactgattg gactaggggt cctcatcgga ttaccgaatt cagtcaaact 960
     ccgaatgcca atgacttatc cttaggagtc agactgcgag tgataagatc cgtagtcaaa 1020
     agggaaacag cccagaccgc cagctaaggt cccaaagtgt gtattaagtg gaaaaggatg 1080
10
     tggagttgct tagacaacta ggatgttggc ttagaagcag ccaccattta aagagtgcgt 1140
     aatagctcac tagtcgagtg actctgcgcc gaaaatgtac cggggctaaa tacaccaccg 1200
     aagctgcgga ttgataccaa tggtatcagt ggtaggggag cgttctaagg acagtgaagt 1260
     cagaccggaa ggactggtgg agtgcttaga agtgagaatg ccggtatgag tagcgaaaga 1320
     cgggtgagaa tcccgtccac cgaatgccta aggtttcctg aggaaggctc gtccgctcag 1380
15
     ggttagtcag gacctaagcc gaggccgaca ggcgtaggcg atggacaaca ggttgatatt 1440
     cctgtaccac ctctttatcg tttgagcaat ggagggacgc agaaggatag aagaagcgtg 1500
     cgattggttg tgcacgtcca agcagttagg ctgataagta ggcaaatccg cttatcgtga 1560
     aggetgaget gtgatgggga ageteettat ggagegaagt etttgattee eegetgeeaa 1620
     gaaaagette tagegagata aaaggtgeet gtacegeaaa eegacacagg taggegagga 1680
20
     gagaatccta aggtgtgcga gagaactctg gttaaggaac tcggcaaaat gaccccgtaa 1740
     cttcgggaga aggggtgctt tcttaacgga aagccgcagt gaataggccc aagcgactgt 1800
     ttagcaaaaa cacagctctc tgcgaagccg taaggcgaag tatagggggt gacacctgcc 1860
     cggtgctgga aggttaagga gaggggttag cgtaagcgaa gctctgaact gaagccccag 1920
     taaacggcgg ccgtaactat aacggtccta aggtagcgaa attccttgtc gggtaagttc 1980
25
     cgacccgcac gaaaggtgta acgatttggg cactgtctca accagagact cggtgaaatt 2040
     atagtacctg tgaagatgca ggttacccgc gacaggacgg aaagaccccg tggagcttta 2100
     ctgtagcctg atattgaatt ttggtacagt ttgtacagga taggcgggag cctttgaaac 2160
     cggagcgcta gcttcggtgg aggcgctggt gggataccgc cctgactgta ttgaaattct 2220
     aacctacggg tcttatcgac ccgggagaca gtgtcaggtg ggcagtttga ctggggcggt 2280
30
    cgcctcctaa agtgtaacgg aggcgcccaa aggttccctc agaatggttg gaaatcattc 2340
    gtagagtgca aaggcataag ggagcttgac tgcgagacct acaagtcgag cagggacgaa 2400
     agtegggett agtgateegg tggtteegea tggaagggee ategeteaac ggataaaage 2460
     taccccgggg ataacaggct tatctccccc aagagtccac atcgacgggg aggtttggca 2520
    cctcgatgtc ggctcatcgc atcctggggc tgtagtcggt cccaagggtt gggctgttcg 2580
35
    cccattaaag cggtacgcga gctgggttca gaacgtcgtg agacagttcg gtccctatcc 2640
    gtcgtgggcg taggaaattt gagaggagct gtccttagta cgagaggacc gggatggacg 2700
     caccgctggt gtaccagttg ttctgccaag ggcatagctg ggtagctatg tgcggaaggg 2760
     ataagtgctg aaagcatcta agcatgaagc ccccctcaag atgagatttc ccatagcgta 2820
     agctagtaag atccctgaaa gatgatcagg ttgataggtt cgaggtggaa gcatggtgac 2880
40
     atgtggagct gacgaatact aatagatcga ggacttaacc at
     <210> 51
     <211> 2912
45
     <212> DNA
     <213> Enterococcus faecalis
     <400> 51
    ggttaagtga ataagggcgc acggtggatg ccttggcact aggagccgat gaaggacggg 60
50
    actaacaccg atatgctttg gggagctgta agtaagctat gatccagaga tttccgaatg 120
    ggggaaccca atatctttta taggatatta cttttcagtg aatacatagc tgattagagg 180
    tagacgcaga gaactgaaac atcttagtac ctgcaggaag agaaagaaaa ttcgattccc 240
    tgagtagegg egagegaaac gggaagagec caaaccaaca agettgettg ttggggttgt 300
    aggactccaa tatggtagtc tgttagtata gttgaaggat ttggaaaatt ccgctaaaga 360
55
    gggtgaaagc cccgtagacg aaatgctaac aacacctagg aggatcctga gtacggcgga 420
    acacgagaaa ttccgtcgga atccgcgggg accatcccgc aaggctaaat actccctagt 480
```

	gaccgatagt	gaaccagtac	cgtgagggaa	aggtgaaaag	caccccggaa	ggggagtgaa	540
	atagatcctg	aaaccgtgtg	cctacaacaa	gtcaaagctc	gttaatgagt	gatggcgtgc	600
		atgaaccggc					
	ccgcagcgaa	agcgagtctg	aatagggcga	atgagtatgt	agtcgtagac	ccgaaaccat	720
5		catgtccagg					
		agtgcgggga					
		ctctccgaaa					
		ttggactagg					
		atatccggga					
10		caccagctaa					
		ctaggatgtt					
		gtgaccctgc					
		attaggtgta					
		ggagcgctta					
15		accgtatgac					
		ccgaggccga					
		gtttgagcaa					
		aagcaatgag					
		agcgaaataa					
20		aaacaactgc					
		gagcgaactc					
		tgacttcggt					
		tgcaaaatcg					
		gatgggttag					
25		aacggtccta					
		acgatttggg					
		ggttacccgc					
	atattgagtg	tttgtaccac	atgtacagga	taggtaggag	ccgatgagac	cggaacgcta	2160
		aggcgctggt					
30		gtgggagaca					
		aggcgcccaa					
	aaggcagaag	ggagcttgac	tgcgagacct	acaagtcgag	cagggacgaa	agtcgggctt	2400
	agtgatccgg	tggttccgca	tggaagggcc	atcgctcaac	ggtaaaagct	accctgggga	2460
	taacaggctt	atctccccca	agagtccaca	tcgacgggga	ggtttggcac	ctcgatgtcg	2520
35		tcctggggct					
	ggcacgcgag	ctgggttcag	aacgtcgtga	gacagttcgg	tccctatccg	tcgcgggcgt	2640
	tggaaatttg	agaggagctg	tccttagtac	gagaggaccg	ggatggactt	accgctggtg	2700
	taccagttgt	tctgccaagg	gcattgctgg	gtagctatgt	agggaaggga	taaacgctga	2760
	aagcatctaa	gtgtgaagcc	cacctcaaga	tgagatttcc	catttcttta	agaaagtaag	2820
40	acccctgaga	gatgatcagg	tagataggtt	ggaagtggaa	ggctagtgat	agttggagcg	2880
	gaccaatact	aatcggtcga	ggacttaacc	aa			2912
					,		
			_				
4.5	<210> 52						
45	<211> 2898						
	<212> DNA						
	<213> Lacto	ococcus lact	is				
	-400- 50						
50	<400> 52	ataaggggg	acout coat c	acttaceset	2202000025	gaaggagete	60
J U		ataagggcgc atattctagg					
		gctgctacta					
		ctgaaacatc gcgaacgcga					
55		ggacttaagc					
J.J.		tagacgaaat					
	aataateeeg	cayacyaaat	agegettata	cccaycayca	cucuyaytay	ggctggacac	42V

```
gcgaaatcca gtttgaatcc gggaggacca tctcccaacc ctaaatactc cttagtgacc 480
    gatagtgaac cagtaccgtg agggaaaggt gaaaagaacc cgagagggga gtgaaatagc 540
     acctgaaacc gtgtgcctac aagaagttcg agcccgttaa tgggtgagag cgtgcctttt 600
    gtagaatgaa ccggcgagtt acgttatgat gcgaggttaa gttgaagaga cggagccgta 660
     gggaaaccga gtctgaatag ggcgacttag tatcatgatg tagacccgaa acctagtgac 720
     ctatccatga gcagggtgaa ggtgtggtaa gacgcactgg aggcccgaac caggacacgt 780
     tgaaaagtgt ttggatgact tgtggatagc ggagaaattc caaacgaact gggagatagc 840
     tggttctctc cgaaatagct ttagggctag cgtcgaaatg taagtgtatt ggaggtagag 900
    cactgtttgg gtgaggggtc cgtctaggat taccaatctc agataaactc cgaatgctaa 960
10
     tacacatgtt cggcagtcag actgcgagtg ctaagatccg tagtcgaaag ggaaacagcc 1020
    cagaccaaca gctaaggtcc caaaatatat gttaagtgga aaaggatgtg gggttgcaca 1080
    gacaactagg atgttagctc agaagcagct atcattcaaa gagtgcgtaa tagctcacta 1140
    gtcgagtgac cctgcgccga aaatgtaccg gggctaaaca tattaccgaa gctttggatt 1200
    qatattttat caatggtagg agagcgttct taaccgcgat gaaggtatac cgtgaggagt 1260
15
    gctggagcgt taagaagtga gaatgccggt atgagtagcg caagataagt gagaatctta 1320
     tecacegtaa gaetaaggtt tecaggggaa ggetegteeg eeetgggtta gtegggaeet 1380
    aaggcgaggc cgaaaggcgt agtcgatgga caactggttg atattccagt actagatatg 1440
    atcgtgatgg agggacgcag taggctaaga gatgccagtt aatggattct ggtctaagca 1500
    gtgaggtgtg agatgtgtca aatgcatttc tctttaacat tgagctgtga tggggaagca 1560
20
    actacggttg cgaactetet gatgteacae tgeeaagaaa agettetage gtaaagteat 1620
    atctacccgt accgcaaacc gacacaggtg gtcgaggcga gtagcctcag gtgatcgaga 1680
    gaactctcgt taaggaactc ggcaaaatag ccccgtaact tcgggagaag gggtgctggt 1740
    gtaaaagcca gccgcagtga ataggcccaa gcaactgttt atcaaaaaca cagctctctg 1800
    ctaaaccgca aggtgatgta tagggggtga cgcctgcccg gtgctggaag gttaagagga 1860
25
    gtgcttagac gtaagtcgaa ggtatgaatt gaagccccag taaacggcgg ccgtaactat 1920
     aacggtccta aggtagcgaa attccttgtc gggtaagttc cgacccgcac gaaaggcgta 1980
     atgatttggg cactgtctca acgagagact cggtgaaatt ttagtacctg tgaagatgca 2040
    ggttacccgc gacaggacgg aaagacccca tggagcttta ctgtagtttg atattgagta 2100
    cctgtaagtc atgtacagga taggtaggag ccattgaaat agggacgcta gtttctattg 2160
30
    aggegttgtt gggatactac cettgactta tggttactct aaccegetgg cataategge 2220
    cagggagaca gtgtctgacg gacagtttga ctggggcggt cgctcctaaa gagtaacgga 2280
    qqcqctcaaa qgttgqctca gattggttgg aaatcaatcg tagagtgtaa aggtaaaagc 2340
    cagettgact gegagageta caactegage aggtaggaaa etaggaetta gtgateeggt 2400
    ggtaccgcat ggaagggcca tcgctcaacg gataaaagct accctgggga taacaggctt 2460.
35
    atotococca agagttcaca togacgggga ggtttggcac ctcgatgtcg gctcgtcgca 2520
     tectgggget gtagteggte ceaagggttg ggetgttege cattaaageg geaegegage 2580
     tgggttcaga acgtcgtgag acagttcggt ccctatccgt cgcgggcgta ggtaatttga 2640
    gaggatetgt cettagtacg agaggacegg gatggactta cegetggtgt accagttgtt 2700
    ccgccaggag cacggctgga tagctatgta gggaagggat aagcgctgaa agcatctaag 2760
40
    tgcgaagccc acctcaagat gagattaccc attcgtaaga attaagagcc cagagagatg 2820
    atctggtaga taggctggaa gtggaagagt tgcgagactt ggagcggacc agtactaatc 2880
    gctcgaggac tttaccaa
45
     <210> 53
     <211> 2932
     <212> DNA
     <213> Listeria monocytogenes
50
     <400> 53
    ggttaagtta gaaagggcgc acggtggatg ccttggcact aggagccgaa gaaggacggg 60
    actaacaccg atatgctttg gggagctgta cgtaagcgtt gatccagaga tttccgaatg 120
    ggggaaccca ctatctttag tcggatagta tccttacgtg aatacatagc gtgaggaagg 180
    cagacccagg gaactgaaac atctaagtac ctggaggaag agaaagaaaa atcgatttcc 240
    tgagtagcgg cgagcgaaac ggaaagagcc caaaccaaga agcttgcttc ttggggttgt 300.
```

aggacactct atacggagtt acaaaagaaa gttataaatg aagcggtctg gaaaggcccg 360

```
ccaaagacgg taacagcccg gtagttgaaa tggctttccc tccagagtgg atcctgagta 420
    cggcggaaca cgtgaaattc cgtcggaatc cgggaggacc atctcccaag gctaaatact 480
    ccctagtgac cgatagtgaa ccagtaccgt gagggaaagg tgaaaagcac cccggaaggg 540
    gagtgaaaca gttcctgaaa ccgtgtgcct acaagtagtt agagcccgtt aatgggtgat 600
    agcgtgcctt ttgtagaatg aaccggcgag ttacgatttg ttgcaaggtt aagcggaaaa 660
    ageggageeg tagegaaage gagtetgaat agggegeata agtaacaggt egtagaceeg 720
    acceacgeae gttgaaaagt geggggatga ggtgtgggta geggagaaat tecaategaa 840
    cttggagata gctggttctc tccgaaatag ctttagggct agcctcgagg taaagagtca 900
10
    tggaggtaga gcactgtttg gactaggggc ccttctcggg ttaccgaatt cagataaact 960
    ccgaatgcca tgtacttata ctcgggagtc agactgcgag tgataagatc cgtagtcgaa 1020
    agggaaacag cccagaccac cagttaaggt ccccaaatat atgttaagtg gaaaaggatg 1080
    tggggttgct tagacaacca ggatgttggc ttagaagcag ccaccattga aagagtgcgt 1140
    aatageteae tggtegagtg acceegegee gaaaatgtae eggggetaaa catattaceg 1200
15 aaactgtgga tgaacctctt tagaggttcg tggtaggaga gcgttctaag ggcggtgaag 1260
    tcagaccgga aggactggtg gagcgcttag aagtgagaat gccggtatga gtagcgaaag 1320
    aagggtgaga atcccttcca ccgaatatct aaggtttcct gaggaaggct cgtccgctca 1380
    qqqttagtcg ggacctaagc cgaggccgat aggcgtaggc gatggacaac aggtagagat 1440
    tcctgtacca gtgctaattg tttaaccgat ggggtgacac agaaggatag ggaatcgcac 1500
20
    gaatggaaat gtgcgtccaa gcagtgagtg tgagaagtag gcaaatccgc ttctcacgaa 1560
    gcatgagctg tgatggggaa ggaaattaag tacggaagtt cctgatttca cgctgtcaag 1620
    aaaagcctct aggaagagta gtactgcccg taccgcaaac cgacacaggt agatgaggag 1680
    agaatcctaa ggtgagcgag agaactctcg ttaaggaact cggcaaaatg accccgtaac 1740
    ttcgggagaa ggggtgctct attagggtgc aagcccgaga gagccgcagt gaataggccc 1800
25
    aggcgactgt ttagcaaaaa cacaggtctc tgcaaaaccg taaggtgacg tataggggct 1860
    gacgcctgcc cggtgctgga aggttaagag gagtgcttag cttcggcgaa ggtacgaatt 1920
    gaagccccag taaacggcgg ccgtaactat aacggtccta aggtagcgaa attccttgtc 1980
    qqqtaaqttc cgacccgcac gaaaggcgca acgatctggg cactgtctca acgagagact 2040
    cggtgaaatt atagtacctg tgaagatgca ggttacccgc gacaggacgg aaagaccccg 2100
30
    tggagcttta ctgcaacctg atatggaatg tttgtaccgc ttgtacagga taggtaggag 2160
    ccgaagagac gtgtgcgcta gcatacgagg aggcaatggt gggatactac cctggctgta 2220
    tgaccattct aacccgccac gcttagcgcg tggggagaca gtgtcaggtg ggcagtttga 2280
    ctggggcggt cgcctcctaa agagtaacgg aggcgcccaa aggttccctc agaatggatg 2340
    gaaatcattc gcagagtgta aaggcacaag ggagcttgac tgcgagactg acaagtcgag 2400
35
    cagggacgaa agtcgggctt agtgatccgg tggttccgca tggaagggcc atcgctcaac 2460
    ggataaaagc taccccgggg ataacaggct tatctccccc aagagtccac atcgacgggg 2520
    aggtttggca cetegatgte ggetegtege atcetgggge tgtagteggt eccaagggtt 2580
    gggctgttcg cccattaaag cggcacgcga gctgggttca gaacgtcgtg agacagttcg 2640
    gtccctatcc gtcgcgggcg caggaaattt gagaggagct gtccttagta cgagaggacc 2700
40
    gggatggaca caccgctggt gtaccagttg ttccgccagg agcatcgctg ggtagctatg 2760
     tgtggcaggg ataaacgctg aaagcatcta agcgtgaagc ccccctcaag atgagatttc 2820
    ccatttcttc ggaaagtaag atccctgaaa gatgatcagg tagataggtt tggagtggaa 2880
    gtgtagcgat acatggagcg gacaaatact aatcgatcga ggacttaacc aa
                                                                     2932
45
     <210> 54
     <211> 2923
     <212> DNA
     <213> Staphylococcus aureus
50
     <400> 54
    gattaagtta ttaagggcgc acggtggatg ccttggcact agaagccgat gaaggacgtt 60
    actaacgacg atatgctttg gggagctgta agtaagcttt gatccagaga tttccgaatg 120
    qqqaaaccca qcatqaqtta tgtcatgtta tcgatatgtg aatacatagc atatcagaag 180
55
    gcacaccegg agaactgaaa catettagta ceeggaggaa gagaaagaaa attegattee 240
     cttagtagcg gcgagcgaaa cgggaagagc ccaaaccaac aagcttgctt gttggggttg 300
```

```
taggacactc tatacggagt tacaaaggac gacattagac gaatcatctg gaaagatgaa 360
    tcaaagaagg taataatcct gtagtcgaaa atgttgtctc tcttgagtgg atcctgagta 420
    cgacggagca cgtgaaattc cgtcggaatc tgggaggacc atctcctaag gctaaatact 480
    ctctagtgac cgatagtgaa ccagtaccgt gagggaaagg tgaaaagcac cccggaaggg 540
    qaqtqaaata gaacctgaaa ccgtgtgctt acaagtagtc agagcccgtt aatgggtgat 600
    qqcqtqcctt ttgtagaatg aaccggcgag ttacgatttg atgcaaggtt aagcagtaaa 660
    tgtggagccg tagcgaaagc gagtctgaat agggcgttta gtatttggtc gtagacccga 720
    aaccaggtga tctacccttg gtcaggttga agttcaggta acactgaatg gaggaccgaa 780
    ccgacttacg ttgaaaagtg agcggatgaa ctgagggtag cggagaaatt ccaatcgaac 840
    ctggagatag ctggttctct ccgaaatagc tttagggcta gcctcaagtg atgattattg 900
10
    gaggtagage actgtttgga cgaggggccc ctctcgggtt accgaattca gacaaactcc 960
    qaatqccaat taatttaact tgggagtcag aacatgggtg ataaggtccg tgttcgaaag 1020
    ggaaacagcc cagaccacca gctaaggtcc caaaatatat gttaagtgga aaaggatgtg 1080
    gcgttgccca gacaactagg atgttggctt agaagcagcc atcatttaaa gagtgcgtaa 1140
15
    tageteacta gtegagtgae actgegeega aaatgtaeeg gggetaaaca tattaeegaa 1200
    gctgtggatt gtcctttgga caatggtagg agagcgttct aagggcgttg aagcatgatc 1260
    gtaaggacat gtggagcgct tagaagtgag aatgccggtg tgagtagcga aagacgggtg 1320
    agaatcccgt ccaccgattg actaaggttt ccagaggaag gctcgtccgc tctgggttag 1380
    tcgggtccta agctgaggcc gacaggcgta ggcgatggat aacaggttga tattcctgta 1440
20
    ccacctataa tegttttaat cgatgggggg acgcagtagg ataggcgaag cgtgcgattg 1500
    gattgcacgt ctaagcagta aggctgagta ttaggcaaat ccggtactcg ttaaggctga 1560
    gctgtgatgg ggagaagaca ttgtgtcttc gagtcgttga tttcacactg ccgagaaaag 1620
    cctctagata gaaaataggt gcccgtaccg caaaccgaca caggtagtca agatgagaat 1680
    tctaaggtga gcgagcgaac tctcgttaag gaactcggca aaatgacccc gtaacttcgg 1740
25
    gagaaggggt gctctttagg gttaacgccc agaagagccg cagtgaatag gcccaagcga 1800
    ctgtttatca aaaacacagg tctctgctaa accgtaaggt gatgtatagg ggctgacgcc 1860
    tgcccggtgc tggaaggtta agaggagtgg ttagcttctg cgaagctacg aatcgaagcc 1920
    ccagtaaacg gcggccgtaa ctataacggt cctaaggtag cgaaattcct tgtcgggtaa 1980
    gttccgaccc gcacgaaagg cgtaacgatt tgggcactgt ctcaacgaga gactcggtga 2040
30
    aatcatagta cctgtgaaga tgcaggttac ccgcgacagg acggaaagac cccgtggagc 2100
    tttactgtag cctgatattg aaattcggca cagcttgtac aggataggta ggagcctttg 2160
    aaacgtgage getagettae gtggaggege tggtgggata etaceetage tgtgttgget 2220
    ttctaacccg caccacttat cgtggtggga gacagtgtca ggcgggcagt ttgactgggg 2280
    cggtcgcctc ctaaaaggta acggaggcgc tcaaaggttc cctcagaatg gttggaaatc 2340
35
    attcatagag tgtaaaggca taagggagct tgactgcgag acctacaagt cgagcagggt 2400
    cgaaagacgg acttagtgat ccggtggttc cgcatggaag ggccatcgct caacggataa 2460
    aagctacccc ggggataaca ggcttatctc ccccaagagt tcacatcgac ggggaggttt 2520
    ggcacctcga tgtcggctca tcgcatcctg gggctgtagt cggtcccaag ggttgggctg 2580
    ttcgcccatt aaagcggtac gcgagctggg ttcagaacgt cgtgagacag ttcggtccct 2640
40
    atccgtcgtg ggcgtaggaa atttgagagg agctgtcctt agtacgagag gaccgggatg 2700
    gacatacete tggtgtacea gttgtegtge caacggeata getgggtage tatgtgtgga 2760
    cgggataagt gctgaaagca tctaagcatg aagcccccct caagatgaga tttcccaact 2820
    tcggttataa gatccctcaa agatgatgag gttaataggt tcgaggtgga agcatggtga 2880
    catgtggagc tgacgaatac taatcgatcg aagacttaat caa
45
    <210> 55
    <211> 2900
    <212> DNA
50
    <213> Streptococcus mutans
    <400> 55
    gttaagttaa taagggcgca cggtggatgc ctaggcacta ggagccgatg aaggacgtga 60
    cgaacgacga catgctttgg ggagctgtaa gtaagccttg atccagagat atccgaatgg 120
    gggaacccaa caggtaatgc ctgttatcca taactgttaa ggttatgaga aggaagacgc 180
55
    agtgaactga aacatctcag tagctgcagg aagagaaagc aagagcgatt gcctcagtag 240
```

```
cggcgagcga agaggcagga gggcaaacca gagtgtttac actctggggt tgtaggactg 300
    cgataaagca gccaagggaa tagaagaaga ctctgggaag agtcgccaga gagagtaaga 360
    gcctcgtatt tgaaattcac ttgatgccaa gcaggatcct gagtacggcg ggacacgagg 420
    aatcccgtcg gaatctggga ggcccatctc ccaaccctaa atactcccta gtgaccgata 480
    gtgaaccagt accgtgaggg aaaggtgaaa agtaccccgg aaggggagtg aaagagaacc 540
    tgaaaccgtg tgcttacaag aagttcgagc ccgttaatgg gtgagagcgt gccttttgta 600
    gaatgaaccg gcgagttacg tttacgtgcg aggttaagtt gaagagacgg agccgtaggg 660
    aaaccgagtc tgaaaagggc ggttaagtac gtagatgtag acccgaaacc aagtgaccta 720
    cccatgagca ggttgaaggt gcggtaaaac gcactggagg accgaaccag gacacgttga 780
10
    aaagtgtttg gatgacttgt gggtagegga gaaattccaa acgaacttgg agatagetgg 840
    ttctctccga aatagcttta gggctagcgt cggtcgcgag actcttggag gtagagcact 900
    gtttgattga ggggtccatc ccggattacc aatctcagat aaactccgaa tgccaacgag 960
    ttaagaccgg cagtcagact gcgagtgcta agatccgtag tcgaaaggga aacagcccag 1020
    accaccaget aaggteecca aataattgtt aagtggaaaa ggatgtgggg ttgcacagae 1080
15
    aactaggatg ttagcttaga agcagctatt cattcaaaga gtgcgtaata gctcactagt 1140
    cgagtgaccc tgcgccgaaa atgtaccggg gctgaaacaa tttaccgaag ctgtggatcc 1200
    cttaggggat ggtaggagag cgttctatgt gcgcagaagg tgtaccgcaa ggagcgctgg 1260
    agtgcataga agtgagaatg ceggtatgag tagegtaaga caggtgagaa teetgteeac 1320
    cgtaagacta aggattccag gggaaggctc gtccgccctg ggttagtcgg gacctaagga 1380
20
    gagaccgata ggtgtatccg atgggcaaca ggttgatatt cctgtactag agtattgagt 1440
    gaaggagga cgcagcaggc taactagagc gtgcgattgg aagagcacgt ccaagcagtg 1500
    aggtgaggac tgagtcaaat gcttagttct gcgccaccaa gctgtgacgg ggagcgaagt 1560
    ttagtagcga agctagtgat gtcactctgc caagaaaagc ttctagcgtt aatgaatact 1620
    ctaccegtac egcaaacega cacaggtagt egaggegagt ageeteaggt gategagega 1680
    actotogtta aggaactogg caaaatggco cogtaactto gggagaaggg gcgctggcga 1740
    taagtcagcc gcagtgaaaa ggcccaagca actgtttatc aaaaacacag ctctctgcga 1800
    aatcgtaaga tgaagtatag ggggtgacgc ctgcccggtg ctggaaggtt aagaggagcg 1860
    cttaqacqtt tgtcgaaggt gtgaattgaa gccccagtaa acggcggccg taactataac 1920
    ggtcctaagg tagcgaaatt ccttgtcggg taagttccga cccgcacgaa aggcgtaatg 1980
30
    atttgggcac tgtctcaacg agagactcgg tgaaatttta gtacctgtga agatgcaggt 2040
    taccegegac aggacggaaa gaccecatgg agetttactg cagtttgata ttgcgtatet 2100
    gttacacatg tacaggatag gtaggagcca aggaagagtg aacgctagtt tacttggagg 2160
    cgttgttggg atactaccct tgtgtgatgg ctactctaac ccggtaggtt gatcatctac 2220
    ggagacagtg tctgacgggc agtttgactg gggcggtcgc ctcctaaagc gtaacggagg 2280
35
    cgcccaaagg ttccctcaga ctggttggaa atcagtcgta gagtgtaaag gtataaggga 2340
    gettgactge gagacagaca agtegageag ggacgaaagt egggettagt gateeggtgg 2400
    taccgtatgg aagggccatc gctcaacgga taaaagctac cctggggata acaggcttat 2460
    ctccccaag agttcacatc gacgggagg tttggcacct cgatgtcggc tcgtcgcatc 2520
    ctggggctgt agtcggtccc aagggttggg ctgttcgccc attaaagcgg cacgcgagct 2580
40
    gggttcagaa cgtcgtgaga cagttcggtc cctatccgtc gcgggcgaag gaaatttgag 2640
    aggatetget cetagtacga gaggaccaga gtggacttac cgctggtgta ccagttgttc 2700
    tgccaagagc atcgctgggt agctaagtag ggaggggata aacgctgaaa gcatctaagt 2760
    gtgaagcccc cctcaagatg agatttccca taacgttcag ttagtaagag ccctgaaaga 2820
    agaacaggta gataggttgg gagtggaagc gttgtgagac gtgaagcgga ccaatactaa 2880
45
                                                                       2900
    tcgctcgagg acttatccaa
    <210> 56
    <211> 2902
50
    <212> DNA
    <213> Streptococcus pneumoniae
    ggttaagtta ataagggcgc acggtggatg ccttggcact aggagccgac gaaggacgtg 60
55
    acaaacgacg atatgccttg ggtagctgta agtaagcgat gatccaggga tttccgaatg 120
    ggggaaccca acaggtaata cctgttaccc acatctgtta aggatgtgag gaggaagacg 180
```

```
cagtgaactg aaacatctaa gtagctgcag gaagagaaag caaaagcgat tgccttagta 240
    gcggcgagcg aaacggcaga agggcaaacc gaagagttta ctcttcgggg ttgtaggact 300
    gcaatgtgga ctcaaagatt atagaagaat gatttgggaa gatcagccaa agagagtaat 360
    agcctcgtat ttaaaatagt ctttgtactt agcagtatcc tgagtacggc gggacacgtg 420
    aaatcccgtc ggaatctggg aggaccatct cccaacccta aatactccct agtgaccgat 480
    agtgaaccag taccgtgagg gaaaggtgaa aagcaccccg ggaggggagt gaaatagaac 540
    ctgaaaccgt gtgcctacaa caagttcgag cccgttaatg ggtgagagcg tgccttttgt 600
    agaatgaacc ggcgagttac gttatgatgc gaggttaagt tgaagagacg gagccgtagg 660
    gaaaccgagt ctgaataggg cgccttagta tcatgacgta gacccgaaac catgtgacct 720
10
    acceatgage aggttgaagg tgeggtaaga egeactggag gacegaacea gggcaegttg 780
    aaaagtgctt ggatgacttg tgggtagcgg agaaattcca aacgaacttg gagatagctg 840
    gttctctccg aaatagcttt agggctagcg tcgacattag agattcttgg aggtagagca 900
    ctqtttqqqt gaggggtcca tcccggatta ccaatctcag ataaactccg aatgccaatg 960
    aattatggtc ggcagtcaga ctgcgagtgc taagatccgt agtcgaaagg gaaacagccc 1020
15
    agaccaccag ctaaggtccc aaaataattg ttaagtggaa aaggatgtgg ggttgcacag 1080
    acaactagga tgttagctta gaagcagcta ttcattcaaa gagtgcgtaa tagctcacta 1140
    gtcgagtgac cctgcgccga aaatgtaccg gggctaaaac aatttaccga agctgtggat 1200
    acctttatag gtatggtagg agagcgttct atgtgtgatg aaggtatacc gtgaggagtg 1260
    ctggaacgca tagaagtgag aatgccggta tgagtagcga aagacaggtg agaatcctgt 1320
20
    ccaccgtaag actaaggttt ccaggggaag gctcgtccgc cctgggttag tcgggaccta 1380
    aggagagacc gaaaggtgta tccgatggac aacaggttga tattcctgta ctagagtatg 1440
    tagtgatgga gggacgcagt aggctaacta aagcagacga ttggaagagt ctgtctaagc 1500
    agtgaggtgt gaattgagtc aaatgcttaa ttctataaca ttgagctgtg atggggagcg 1560
    aagtttagta gcgaagttag tgacgtcaca ctgccaagaa aagcttctag cgtttaaaca 1620
25
    tactctaccc gtaccgcaaa ccgacacagg tagtcgaggc gagtagcctc aggtgagcga 1680
    gagaactete gttaaggaac teggeaaaat gacceegtaa ettegggaga aggggtgetg 1740
    acttaaagtc agccgcagtg aataggccca agcaactgtt tatcaaaaac acagctctct 1800
    gctaaatcgt aagatgatgt atagggggtg acgcctgccc ggtgctggaa ggttaagagg 1860
    agtgcttagc gtaagcgaag gtatgaattg aagccccagt aaacggcggc cgtaactata 1920
30
    acggtcctaa ggtagcgaaa ttccttgtcg ggtaagttcc gacccgcacg aaaggcgtaa 1980
    tgatttgggc actgtctcaa cgagagactc ggtgaaattt tagtacctgt gaagatgcag 2040
    gttacccgcg acaggacgga aagaccccat ggagctttac tgcagtttga tattgagtgt 2100
    ctgtaccaca tgtacaggat aggtaggagt ctaagagatc gggacgccag tttcgaagga 2160
    gacgctgttg ggatactacc cttgtgttat ggccactcta acccagatag gtgatcccta 2220
35
    teggagacag tgtetgaegg geagtttgae tggggeggte geeteetaaa aggtaaegga 2280
    ggcgcccaaa ggttccctca gaatggttgg aaatcattcg cagagtgtaa aggtataagg 2340
    gagettgaet gegagageta caactegage agggaegaaa gtegggetta gtgateeggt 2400
    ggtteegtat ggaagggeea tegeteaacg gataaaaget accetgggga taacaggett 2460
    atetececca agagtteaca tegaegggga ggtttggcae etegatgteg getegtegca 2520
40
    tectgggget gtagteggte ccaagggttg ggetgttege ccattaaage ggeaegegag 2580
    ctgggttcag aacgtcgtga gacagttcgg tccctatccg tcgcgggcgt aggaaatttg 2640
     agaggatetg etectagtae gagaggaeea gagtggaett accgetggtg taccagttgt 2700
     gtgtgaaacc cacctcaaga tgagatttcc catgattata tatcagtaag agccctgaga 2820
45
    gatgatcagg tagataggtt agaagtggaa gtgtggcgac acatgtagcg gactaatact 2880
                                                                     2902
     aatagctcga ggacttatcc aa
     <210> 57
50
     <211> 2901
     <212> DNA
     <213> Streptococcus pyogenes
     <400> 57
```

ggttaagtta ataagggcgc acggtggatg ccttggcact agaagccgaa gaaggacgtg 60 actaacgacg aaatgctttg gggagctgta agtaagcgct gatccagaga tgtccgaatg 120

55

```
ggggaacccg gcatgtaatg catgtcatcc atgactgtta aggtcatgag aaggaagacg 180
    caqtgaactg aaacatctaa gtagctgcag gaagagaaag caaacgcgat tgccttagta 240
    gcggcgagcg aaacggcagg agggcaaacc gaggagttta ctcctcgggg ttgtaggact 300
    gcgaagtggg acataaagtt aatagaagaa ttacctggga aggtaagcca aagagagtaa 360
    cagectegta tttaaaattg actttageee tageagtate etgagtaegg egagaeaege 420
    gaaatctcgt cggaatctgg gaggaccatc tcccaaccct aaatactctc tagtgaccga 480
    cctgaaaccg tgtgcctaca acaagttcga gcccgttaat gggtgagagc gtgccttttg 600
    tagaatgaac cggcgagtta cgatatgatg cgaggttaag ttgaagagac ggagccgtag 660
10
    ggaaaccgag tottaatagg gcgtcatagt atcatgttgt agacccgaaa ccatgtgacc 720
    tacccatgag caggttgaag gtgtggtaaa acgcactgga ggaccgaacc agggcacgtt 780
    gaaaagtgct tggatgactt gtgggtagcg gagaaattcc aaacgaactt ggagatagct 840
    qqttctctcc gaaatagctt tagggctagc gtcgatgtta agtctcttgg aggtagagca 900
    ctgtttgggt gaggggtcca tcccggatta ccaatctcag ataaactccg aatgccaacg 960
15
    agatataatc ggcagtcaga ctgcgagtgc taagatccgt agtcgaaagg gaaacagccc 1020
    agaccaccag ctaaggtccc aaaataactg ttaagtggaa aaggatgtgg ggttgcacag 1080
    acaactagga tgttagctta gaagcagcta ttcattcaaa gagtgcgtaa tagctcacta 1140
    gtcgagtgac cctgcgccga aaatgtaccg gggctaaaac agtttaccga agctgtggat 1200
    gacacaaaag tgtcatggta ggagagcgtt ctatgtgtga agaaggtgta ccgtgaggag 1260
20
    cgctggaacg catagaagtg agaatgccgg tatgagtagc gaaagacagg tgagaatcct 1320
    gtccaccgta agactaaggt ttccagggga aggctcgtcc gccctgggtt agtcgggacc 1380
    taaggagaga ccgaaaggtg tatccgatgg ccaacaggtt gatattcctg tactagagta 1440
    tatagtgatg gagggacgca gtaggctaac taaaccggac gattggaaga gtccggctaa 1500
    gcagtgaggt gtaagatgag tcaaatgctt atctttataa cattgagctg tgatggggag 1560
25
    cgaattttag tagcgaagtt agtgatgtca cactgccaag aaaagcttct agcgtttaat 1620
    gatactetac cegtacegea aacegacaca ggtagtegag gegagtagee teaggtgate 1680
    gagagaactc tcgttaagga actcggcaaa atgaccccgt aacttcggga gaaggggtgc 1740
    tgacttaggt cagccgcagt gaataggccc aagcaactgt ttatcaaaaa cacagctctc 1800
    tgctaaatcg taagatgatg tatagggggt gacgcctgcc cggtgctgga aggttaagag 1860
    gagggtttag cgcaagcgaa gatctgaatt gaagccccag taaacggcgg ccgtaactat 1920
    aacggtccta aggtagcgaa attccttgtc gggtaagttc cgacccgcac gaaaggcgta 1980
    atgatttggg cactgtctca acgagagact cggtgaaatt ttagtacctg tgaagatgca 2040
    ggttacccgc gacaggacgg aaagacccca tggagcttta ctgcagtttg atattgagta 2100
    tctgtaccac atgtacagga taggtaggag ccattgactt cgggacgcca gtttcgaatg 2160
35
    aggcgttgtt gggatactac ccttgtgtta tggctactct aacccagata ggttatccct 2220
    atcggagaca gtgtctgacg ggcagtttga ctggggcggt cgcctcctaa agagtaacgg 2280
    aggegeceaa aggtteeete agattggttg gaaatcaate geagagtgta aaggtataag 2340
    ggagettgae tgegagaget acaactegag cagggaegaa agtegggett agtgateegg 2400
    tggtaccgaa tggaagggcc atcgctcaac ggataaaagc taccctgggg ataacaggct 2460
    tatetecece aagagtteae ategaegggg aggtttggea cetegatgte ggetegtege 2520
    atcctggggc tgtagtcggt cccaagggtt gggctgttcg cccattaaag cggcacgcga 2580
    gctgggttca gaacgtcgtg agacagttcg gtccctatcc gtcgcgggcg taggaaattt 2640
    gagaggatet geteetagta egagaggaee agagtggaet tacegetggt gtaceagttg 2700
    tcttgccaaa ggcatcgctg ggtagctatg tagggaaggg ataagcgctg aaagcatcta 2760
45
    agtgcgaagc ccccctcaag atgagatttc ccatgatttt atatcagtaa gagccctgag 2820
    agatgatcag gtagataggt taggagtgta agtgtagcga tacatgtagc ggactaatac 2880
                                                                     2901
    taatagctcg aggacttatc c
```

50 <210> 58

<211> 3107

<212> DNA

<213> Mycobacterium avium

55

tgtgtgtaag taagtgttta agggcgcatg gtggatgcct tggcatcgag agccgatgaa 60

	ggacgtggga	ggctgcgata	tgcctcgggg	agctgtcaac	cgagcattga	tccgaggatt	120
	tccgaatggg.	ggaacccagc	acgagtgatg	tcgtgttacc	cgtatctgaa	tatatagggt	180
	gcgggaggta	acgcggggaa	gtgaaacatc	tcagtacccg	taggagaaga	aaacaattgt	240
	gattccgtca	gtagtggcga	gcgaaccgga	acaggctaaa	ccgcatgcat	ggacaaccgg	300
5	gtaggggttg	tgtgtgcggg	gttgtgggat	tgatatgtct	cagctctacc	tggctgaggg	360
	gtagtcagaa	agtgtcgtgg	ttagcggaag	tggcctggga	cggcccgccg	tagacggtga	420
	gagcccggta	cgcgaaaacc	cggcacctgc	cttatatcaa	cacccgagta	gcagcgggcc	480
	cgtggaatct	gctgtgaatc	tgccgggacc	acccggtaag	cctaaatact	tctcgatgac	540
	cgatagcgga	ttagtaccgt	gagggaatgg	tgaaaagtac	cccgggaggg	agtgaaatag	600
10	tacctgaaac	cgtgtgccta	caatccgtca	gagcctcctc	gtggggtgat	ggcgtgcctt	660
	ttgaagaatg	agcctgcgag	tcagggacac	gtcgcgaggt	taacccgtgc	ggggtagccg	720
	cagcgaaagc	gagtctgaat	agggcgcatc	ccctttgggg	tgtagtggcg	tgttctggac	780
	ccgaagcgga	gtgatctacc	catggccagg	gtgaagcgcg	ggtaagaccg	cgtggaggcc	840
	cgaacccact	taggttgaag	actgagggga	tgagctgtgg	gtaggggtga	aaggccaatc	900
15	aaactccgtg	atagctggtt	ctccccgaaa	tgcatttagg	tgcagcgttg	cgtggttcac	960
	cacggaggta	gagctactgg	atggccgatg	ggccctacta	ggttactgac	gtcagccaaa	1020
	ctccgaatgc	cgtggtgtaa	aagcgtggca	gtgagacggc	gggggataag	ctccgtacgt	1080
	cgaaagggaa	acagcccaga	tcgccggcta	aggcccctaa	gcgtgtgcta	agtggaaaag	1140
	gatgtgtagt	cgcagagaca	accaggaggt	tggcttagaa	gcagccatcc	ttgaaagagt	1200
20	gcgtaatagc	tcactggtca	agtgattatg	cgccgataat	gtagcggggc	tcaagcacac	1260
	cgccgaagcc	gcggcacatt	catctttacg	gtggatgtgg	gtaggggagc	gtcccccatt	1320
	cagcgaagct	ccgggtgacc	ggtggtggag	ggtgggggag	tgagaatgca	ggcatgagta	1380
	gcgataaggc	aagtgagaac	cttgcccgcc	gtaagaccaa	gggttcctgg	gccaggccag	1440
	tccgcccagg	gtgagtcggg	acctaaggcg	aggccgacag	ggtagtcgat	ggacaacggg	1500
25	ttgatattcc	cgtacccgtg	tatgggcgtc	cctgatgaat	cagcggtact	aaccacccaa	1560
	aaccggatcg	accattcccc	ttcgggggcg	tggcgattcg	gggctgcgtg	ggaccttcgc	1620
	tggtagtagt	caagcaatgg	ggtgacgcag	gaaggcagcc	gtaccagtca	gtggtaatac	1680
	tggggcaagc	ccgtagagag	cgataggcaa	atccgtcgct	cactaatcct	gagaggtgat	1740
	gcatagccgg	ttgaggcgaa	ttcggtgatc	ctctgctgcc	aagaaaagcc	tctagcgagc	1800
30	acatacacgg	cccgtacccc	aaaccaacac	aggtggtcag	gtagagaata	ccaaggcgta	1860
	cgagataact	atggttaagg	aactcggcaa	aatgcccccg	taacttcggg	agaagggggc	1920
	ccggaatacc	gtgaacaccc	ttgcggtggg	agcgggattc	ggccgcagaa	accagtgggt	1980
	agcgactgtt	tactaaaaac	acaggtccgt	gcgaagtcgc	aagacgatgt	atacggactg	2040
	acgcctgccc	ggtgctggaa	ggttaagagg	acccgttaac	ccgtaagggt	gaagcggaga	2100
35	atttaagccc	cagtaaacgg	cggtggtaac	tataaccatc	ctaaggtagc	gaaattcctt	2160
	gtcgggtaag	ttccgacctg	cacgaatggc	gtaacgactt	cccaactgtc	tcaaccatag	2220
	actcggcgaa	attgcactac	gagtaaagat	gctcgttacg	cgcggcagga	cgaaaagacc	2280
	ccgggacctt	cactacaact	tggtattggt	gttcggtacg	gtttgtgtag	gataggtggg	2340
	agactttgaa	gcacagacgc	cagtttgtgt	ggagtcgttg	ttgaaatacc	actctgatcg	2400
40	tattggacac	ctaacgtcga	acccttatcg	ggttcacgga	cagtgcctgg	cgggtagttt	2460
	aactggggcg	gttgcctcct	aaaatgtaac	ggaggcgccc	aaaggttccc	tcaacctgga	2520
	cggcaatcag	gtggcgagtg	taagtgcaca	agggagcttg	actgcgagac	ttacaagtca	2580
	agcagggacg	aaagtcggga	ctagtgatcc	ggcacccccg	agtggaaggg	gtgtcactca	2640
	acggataaaa	ggtaccccgg	ggataacggg	ctgatcttcc	ccaagagtcc	atatcgacgg	2700
45	gatggtttgg	cacctcgatg	tcggctcgtc	gcatcctggg	gctggagcag	gtcccaaagg	2760
•	ttgggctgtt	cgcccattaa	agcggcacgc	gagctgggtt	tagaacgtcg	tgagacagtt	2820
	cggtctctat	ccgccgcgcg	cgtcagaaac	ttgaggaaac	ctgtccctag	tacgagagga	2880
	ccgggacgga	cgaacctctg	gtataccagt	tgtcccacca	ggggcacggc	tggatagcca	2940
	cgttcggaca	ggataaccgc	tgaaagcatc	taagcgggaa	accttctcca	agatcaggtt	3000
50	tctcaccctt	ttagagggat	aaggcccccc	gcagaccacg	ggattgatag	gccagacctg	3060
	gaagctcagt	aatgagtgca	gggaactggc	actaactggc	cgaaagc		3107

<210> 59 55 <211> 3138 <212> DNA

<213> Mycobacterium tuberculosis

```
<400> 59
    ttgtaagtgt ctaagggcgc atggtggatg ccttggcatc gagagccgat gaaggacgtg 60
    ggaggetgeg atatgeeteg gggagetgte aacegagegt ggateegagg attteegaat 120
    ggggaaaccc agcacgagtg atgtcgtgct acccgcatct gaatatatag ggtgcgggag 180
    ggaacgeggg gaagtgaaac atctcagtac cegtaggagg agaaaacaat tgtgatteeg 240
    caagtagtgg cgagcgaacg cggaacaggc taaaccgcac gcatgggtaa ccgggtaggg 300
    gttgtgtgtg cggggttgtg ggaggatatg tctcagcgct acccggctga gaggcagtca 360
10
    gaaagtgtcg tggttagcgg aagtggcctg ggatggtctg ccgtagacgg tgagagcccg 420
    gtacgcgaaa acccggcacc tgcctagtat caattcccga gtagcagcgg gcccgtggaa 480
    tccgctgtga atccgccggg accacccggt aagcctaaat actcctcgat gaccgatagc 540
    ggattagtac cgtgagggaa tggtgaaaag taccccggga ggggagtgaa agagtacctg 600
    aaaccgtgtg cctacaatcc gtcagagcct ccttttcctc tccggaggag ggtggtgatg 660
15
    gcgtgccttt tgaagaatga gcctgcgagt cagggacatg tcgcaaggtt aacccgtgtg 720
    gggtagccgc agcgaaagcg agtctgaata gggcgaccca cacgcgcata cgcgcgtgtg 780
    aatagtggcg tgttctggac ccgaagcgga gtgatctacc catggccagg gtgaagcgcg 840
    ggtaagaccg cgtggaggcc cgaacccact taggttgaag actgagggga tgagctgtgg 900
    gtaggggtga aaggccaatc aaactccgtg atagctggtt ctccccgaaa tgcatttagg 960
20
    tgcagcgttg cgtggttcac cgcggaggta gagctactgg atggccgatg ggccctacta 1020
    ggttactgac gtcagccaaa ctccgaatgc cgtggtgtaa agcgtggcag tgagacggcg 1080
    ggggataagc teegtaegte gaaagggaaa cageecagat egeeggetaa ggeececaag 1140
    cgtgtgctaa gtgggaaagg atgtgcagtc gcaaagacaa ccaggaggtt ggcttagaag 1200
    cagccaccct tgaaagagtg cgtaatagct cactggtcaa gtgattgtgc gccgataatg 1260
25
    tagcgggget caagcacacc gccgaagccg cggcacatcc accttgtggt gggtgtgggt 1320
    aggggagcgt ccctcattca gcgaagccac cgggtgaccg gtggtggagg gtgggggagt 1380
    gagaatgcag gcatgagtag cgacaaggca agtgagaacc ttgcccgccg aaagaccaag 1440
    ggtteetggg ceaggeeagt eegeeeaggg tgagteggga eetaaggega ggeegaeagg 1500
    cgtagtcgat ggacaacggg ttgatattcc cgtacccgtg tgtgggcgcc cgtgacgaat 1560
30
    cagcggtact aaccaccaa aaccggatcg atcactcccc ttcgggggtg tggagttctg 1620
    gggctgcgtg ggaacttcgc tggtagtagt caagcgaagg ggtgacgcag gaaggtagcc 1680
    gtaccagtca gtggtaacac tggggcaagc cggtagggag agcgataggc aaatccgtcg 1740
    ctcactaatc ctgagaggtg acgcatagcc ggttgaggcg aattcggtga tcctctgctg 1800
    ccaagaaaag cctctagcga gcacacacac ggcccgtacc ccaaaccgac acaggtggtc 1860
35
    aggtagagca taccaaggcg tacgagataa ctatggttaa ggaactcggc aaaatgcccc 1920
    cgtaacttcg ggagaagggg gaccggaata tcgtgaacac ccttgcggtg ggagcgggat 1980
    ccggtcgcag aaaccagtga ggagcgactg tttactaaaa acacaggtcc gtgcgaagtc 2040
    gcaagacgat gtatacggac tgacgcctgc ccggtgctgg aaggttaaga ggacccgtta 2100
    accegeaagg gtgaagegga gaatttaage eecagtaaac ggeggtggta actataacca 2160
40
    tcctaaggta gcgaaattcc ttgtcgggta agttccgacc tgcacgaatg gcgtaacgac 2220
    ttctcaactg tctcaaccat agactcggcg aaattgcact acgagtaaag atgctcgtta 2280
    cgcgcggcag gacgaaaaga ccccgggacc ttcactacaa cttggtattg atgttcggta 2340
    eggtttgtgt aggataggtg ggagactgtg aaacetegae gecagttggg geggagtegt 2400
    tgttgaaata ccactctgat cgtattgggc atctaacctc gaaccctgaa tcgggtttag 2460
45
    ggacagtgcc tggcgggtag tttaactggg gcggttgcct cctaaaatgt aacggaggcg 2520
    cccaaaggtt ccctcaacct ggacggcaat caggtggcga gtgtaaatgc acaagggagc 2580
    ttgactgcga gacttacaag tcaagcaggg acgaaagtcg ggattagtga tccggcaccc 2640
    ccgagtggaa ggggtgtcgc tcaacggata aaaggtaccc cggggataac aggctgatct 2700
    tececaagag tecatatega egggatggtt tggcaceteg atgteggete gtegeateet 2760
50
    ggggctggag caggtcccaa gggttgggct gttcgcccat taaagcggca cgcgagctgg 2820
    gtttagaacg tegtgagaca gtteggtete tateegeege gegegteaga aacttgagga 2880
    aacctgtccc tagtacgaga ggaccgggac ggacgaacct ctggtgcacc agttgtcccg 2940
    ccaggggcac cgctggatag ccacgttcgg tcaggataac cgctgaaagc atctaagcgg 3000
    gaaaccttct ccaagatcag gtttctcacc cacttggtgg gataaggccc cccgcagaac 3060
55
    acgggttcaa taggtcagac ctggaagctc agtaatgggt gtagggaact ggtgctaacc 3120
                                                                       3138
    ggccgaaaac ttacaaca
```

```
<210> 60
    <211> 2903
    <212> DNA
     <213> Escherichia coli
     <400> 60
    ggttaagcga ctaagcgtac acggtggatg ccctggcagt cagaggcgat gaaggacgtg 60
    ctaatctgcg ataagcgtcg gtaaggtgat atgaaccgtt ataaccggcg atttccgaat 120
10
    ggggaaaccc agtgtgattc gtcacactat cattaactga atccataggt taatgaggcg 180
    aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattccccc 240
    agtagcggcg agcgaacggg gaggagccca gagcctgaat cagtgtgtgt gttagtggaa 300
    gcgtctggaa aggcgcgcga tacagggtga cagccccgta cacaaaaatg cacatactgt 360
15
    gagetegatg agtagggegg gacacgtggt atcetgtetg aatatggggg gaccateete 420
    caaggctaaa tactcctgac tgaccgatag tgaaccagta ccgtgaggga aaggcgaaaa 480
    gaaccccggc gaggggagtg aaaaagaacc tgaaaccgtg tacgtacaag cagtgggagc 540
    ctcttttatg gggtgactgc gtaccttttg tataatgggt cagcgactta tattctgtag 600
    caaggttaac cgaatagggg agccgaaggg aaaccgagtc ttaaccgggc gttaagttgc 660
    agggtataga cccgaaaccc ggtgatctag ccatgggcag gttgaaggtt gggtaacact 720
20
    aactggagga ccgaaccgac taatgttgaa aaattagcgg atgacttgtg gctgggggtg 780
    aaaggccaat caaaccggga gatagctggt tctccccgaa agctatttag gtagcgcctc 840
    qtqaattcat ctccgggggt agagcactgt ttcggcaagg gggtcatccc gacttaccaa 900
    cccgatgcaa actgcgaata ccggagaatg ttatcacggg agacatacgg cgggtgctaa 960
25
    cgtccgtcgt gaagagggaa acaacccaga ccgccagcta aggtcccaaa gtcatggtta 1020
     agtgggaaac gatgtgggaa ggcccagaca gccaggatgt tggcttagaa gcagccatca 1080
     tttaaagaaa gcgtaatagc tcactggtcg agtcggcctg cgcggaagat gtaacggggc 1140
    taaaccatgc accgaagctg cggcagcgac actgtgtgtt gttgggtagg ggagcgttct 1200
    gtaagcctgt gaaggtgtac tgtgaggtat gctggaggta tcagaagtgc gaatgctgac 1260
    ataagtaacg ataaagcggg tgaaaagccc gctcgccgga agaccaaggg ttcctgtcca 1320
30
    acgttaatcg gggcagggtg agtcgacccc taaggcgagg ccgaaaggcg tagtcgatgg 1380
    gaaacaggtt aatattcctg tacttggtgt tactgcgaag gggggacgga gaaggctatg 1440
    ttggccgggc gacggttgtc ccggtttaag cgtgtaggct ggttttccag gcaaatccgg 1500
    aaaatcaagg ctgaggcgtg atgacgaggc actacggtgc tgaagcaaca aatgccctgc 1560
35
     ttccaggaaa agcctctaag catcaggtaa catcaaatcg taccccaaac cgacacaggt 1620
    ggtcaggtag agaataccaa ggcgcttgag agaactcggg tgaaggaact aggcaaaatg 1680
    gtgccgtaac ttcgggagaa ggcacgctga tatgtaggtg aagtccctcg cggatggagc 1740
     tgaaatcagt cgaagatacc agctggctgc aactgtttat taaaaacaca gcactgtgca 1800
    aacacgaaag tggacgtata cggtgtgacg cctgcccggt gccggaaggt taattgatgg 1860
40
    ggtcagcgca agcgaagctc ttgatcgaag ccccggtaaa cggcggccgt aactataacg 1920
    gtcctaaggt agcgaaattc cttgtcgggt aagttccgac ctgcacgaat ggcgtaatga 1980
     tggccaggct gtctccaccc gagactcagt gaaattgaac tcgctgtgaa gatgcagtgt 2040
     accegeggea agaeggaaag acceegtgaa cetttaetat agettgacae tgaacattga 2100
    gccttgatgt gtaggatagg tgggaggctt tgaagtgtgg acgccagtct gcatggagcc 2160
45
     gaccttgaaa taccaccctt taatgtttga tgttctaacg tggacccgtg atccgggttg 2220
     cggacagtgt ctggtgggta gtttgactgg ggcggtctcc tcctaaagag taacggagga 2280
     gcacgaaggt tggctaatcc tggtcggaca tcaggaggtt agtgcaatgg cataagccag 2340
     cttgactgcg agcgtgacgg cgcgagcagg tgcgaaagca ggtcatagtg atccggtggt 2400
     tctgaatgga agggccatcg ctcaacggat aaaaggtact ccggggataa caggctgata 2460
50
     ccgcccaaga gttcatatcg acggcggtgt ttggcacctc gatgtcggct catcacatcc 2520
     tggggctgaa gtaggtccca agggtatggc tgttcgccat ttaaagtggt acgcgagctg 2580
    ggtttagaac gtcgtgagac agttcggtcc ctatctgccg tgggcgctgg agaactgagg 2640
    ggggctgctc ctagtacgag aggaccggag tggacgcatc actggtgttc gggttgtcat 2700
    gccaatggca ctgcccggta gctaaatgcg gaagagataa gtgctgaaag catctaagca 2760
55
     cgaaacttgc cccgagatga gttctccctg accctttaag ggtcctgaag gaacgttgaa 2820
     gacgacgacg ttgataggcc gggtgtgtaa gcgcagcgat gcgttgagct aaccggtact 2880
```

PCT/US02/41014

```
2903
    aatgaaccgt gaggcttaac ctt
    <210> 61
     <211> 2903
     <212> DNA
     <213> Klebsiella pneumoniae
    <400> 61
    ggttaagcga ctaagcgtac acggtggatg ccctggcagt cagaggcgat gaaggacgtg 60
10
    ctaatctgcg aaaagcgtcg gtaaggtgat atgaaccgtt ataaccggcg atgtccgaat 120
    qqqqaaaccc agtgcaattc gttgcactat cgttaactga atacataggt taacgaggcg 180
    aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattccccc 240
    agtagcggcg agcgaacggg gagcagccca gagtctgaat cagcttgtgt gttagtggaa 300
15
    cggtctggaa agtccgacgg tacagggtga tagtcccgta caccaaaatg cacaggctgt 360
    gaactcgaag agtagggcgg gacacgtggt atcctgtctg aatatggggg gaccatcctc 420
    caaggctaaa tactcctgac tgaccgatag tgaaccagta ccgtgaggga aaggcgaaaa 480
    gaaccccggc gaggggagtg aaaaagaacc tgaaaccgtg tacgtacaag cagtgggagc 540
    accttcgggt gtgactgcgt accttttgta taatgggtca gcgacttata ttctgtagca 600
    aggttaaccg tataggggag ccgcagggaa accgagtctt aactgggcgt taagttgcag 660
20
    ggtatagacc cgaaacccgg tgatctagcc atgggcaggt tgaaggttgg gtaacactaa 720
     ctggaggacc gaaccgacta atgttgaaaa attagcggat gacttgtggc tgggggtgaa 780
     aggccaatca aaccgggaga tagctggttc tccccgaaag ctatttaggt agcgcctcgt 840
    gaactcatct tcgggggtag agcactgttt cggctagggg gtcatcccga cttaccaacc 900
     cgatgcaaac tacgaatacc gaagaatgtt atcacgggag acacacggcg ggtgctaacg 960
25
     tccgtcgtga agagggaaac aacccagacc gccagctaag gtcccaaagt catggttaag 1020
     tgggaaacga tgtgggaagg cacagacagc caggatgttg gcttagaagc agccatcatt 1080
     taaagaaagc gtaatagctc actggtcgag tcggcctgcg cggaagatgt aacggggcta 1140
     aaccatgcac cgaagctgcg gcagcgacac tatgtgttgt tgggtagggg agcgttctgt 1200
     aagcctgcga aggtgtgctg tgaggcatgc tggaggtatc agaagtgcga atgctgacat 1260
30
     aagtaacgat aaagcgggtg aaaagcccgc tcgccggaag accaagggtt cctgtccaac 1320
     gttaatcggg gcagggtgag tcgacccta aggcgaggcc gaaaggcgta gtcgatggga 1380
     aacaggttaa tattcctgta cttggtgtta ctgcgaaggg gggacggaga aggctatgtt 1440
     agccgggcga cggttgtccc ggtttaagca tgtaggctgg ttgtccaggc aaatccggat 1500
     aatcaaggct gaggtgtgat gacgaggcac tacggtgctg aagtaacaaa tgctctgctt 1560
35
     ccaqqaaaaq cctctaagca tcaggtaaca tcaaatcgta ccccaaaccg acacaggtgg 1620
     tcaggtagag aataccaagg cgcttgagat aactcgggtg aaggaactag gcaaaatggt 1680
     gccgtaactt cgggagaagg cacgctggtg tgtaggtgaa gcccctgccg ggtggagctg 1740
     agaccagtcg aagataccag ctggctgcaa ctgtttatta aaaacacagc actgtgcaaa 1800
     cacgaaagtg gacgtatacg gtgtgacgcc tgcccggtgc cggaaggtta attgatgggg 1860
40
     ttatccgtaa ggagaagctc ttgatcgaag ccccggtaaa cggcggccgt aactataacg 1920
     gtcctaaggt agcgaaattc cttgtcgggt aagttccgac ctgcacgaat ggcgtaatga 1980
     tggccaggct gtctccaccc gagactcagt gaaattgaac tcgctgtgaa gatgcagtgt 2040
     acccgcggca agacggaaag accccgtgaa cctttactat agcttgacac tgaacattga 2100
     gccttgatgt gtaggatagg tgggaggctt tgaagcgtgg acgccagtct gcgtggagcc 2160
45
     aaccttgaaa taccaccctt taatgtttga tgttctaacg ttggcccctc accggggttg 2220
     cggacagtgt ctggtgggta gtttgactgg ggcggtctcc tcccaaagcg taacggagga 2280
     gcacgaaggt tagctaatcc tggtcggaca tcaggaggtt agtgcaatgg cataagctag 2340
     cttgactgcg agcgtgacgg cgcgagcagg tgcgaaagca ggtcatagtg atccggtggt 2400
     tctgaatgga agggccatcg ctcaacggat aaaaggtact ccggggataa caggctgata 2460
50
     ccgcccaaga gttcatatcg acggcggtgt ttggcacctc gatgtcggct catcacatcc 2520
     tggggctgaa gtaggtccca agggtatggc tgttcgccat ttaaagtggt acgcgagctg 2580
     ggtttagaac gtcgtgagac agttcggtcc ctatctgccg tgggcgctgg agaattgagg 2640
     ggggctgctc ctagtacgag aggaccggag tggacgcatc actggtgttc gggttgtcat 2700
55
     gccaatggca ctgcccggta gctaaatgcg gaagagataa gtgctgaaag catctaagca 2760
     cgaaacttgc cccgagatga gttctccctg agactttaag tctcctgaag gaacgttgaa 2820
```

qacqacqacg ttgataggcc gggtgtgtaa gcgcagcgat gcgttgagct aaccggtact 2880 aatgaaccgt gaggcttaac ctt 5 <210> 62 <211> 2897 <212> DNA <213> Haemophilus influenzae 10 <400> 62 gtatagttaa gtgactaagc gtacaaggtg gatgccttgg caatcagagg cgaagaagga 60 cgtgctaatc tgcgaaaagc ttggatgagt cgataagagg cgtttaatcc aagatatccg 120 aatggggaaa cccagtagat gaagaatcta ctatcaacaa gtgaattcat agcttgttga 180 ggcaaaccgg gagaactgaa acatctaagt accccgagga aaagaaatca accgagattt 240 cgtcagtagc ggcgagcgaa agcgaaagag ccagtaagtg atagcaatat agtgaggaga 300 atgtgttggg aagcacaatc aaagagggtg ataatcccgt atctaaaaac catattgtgg 360 tactaagcta acgagaagta gggcgggaca cgtgatatcc tgtttgaaga aggggggccc 420 atcctccaaq qctaaatact cctgattgac cgatagtgaa ccagtactgt gaaggaaagg 480 cgaaaagaac cccggtgagg ggagtgaaat agaacctgaa accttgtacg tacaagcagt 540 gggagcgagg gcaaccttgt gactgcgtac cttttgtata atgggtcagc gacttatatt 600 20 ttgtagcgag gttaaccgaa taggggagcc gaagggaaac cgagtcttaa ctgggcgaat 660 agttgcaagg tatagacccg aaacccggtg atctagccat gggcaggttg aaggttgggt 720 aacactaact ggaggaccga accgactaat gttgaaaaat tagcggatga cttgtggctg 780 ggggtgaaag gccaatcaaa ccgggagata gctggttctc cccgaaatct atttaggtag 840 25 agecttgagg tgacacettt gggggtagag cactgttteg getaggggge catecegget 900 taccaacccq atgcaaacta cgaataccaa agagtgatac tcaggagaca cacggcgggt 960 gctaacgtcc gtcgtggaga gggaaacaac ccagaccgcc agctaaggtc cccaagtcta 1020 tattaagtgg gaaacgaagt gggaaggctt agacagctag gatgttggct tagaagcagc 1080 30 ggggctgaaa tatagcaccg aagctgcggc atcagaattt attctgttgg gtaggggagc 1200 gttgtgtaag cggaagaagg ttcatcgaga ggtgggctgg acgtatcaca agtgcgaatg 1260 ctgacataag taacgataaa acgggtgaaa aacccgttcg ccggaagacc aagggttcct 1320 gtccaacgtt aatcggggca gggtgagtcg gctcctaagg cgaggctgaa aagcgtagtc 1380 qatqqqaaac aggttaatat tcctgtactt ggtaaagctg cgatgtgggg acggagtagg 1440 35 ttaggttatc gcactgttgg atatgtgcgt ttaagttggt aggtgggaag tttaggcaaa 1500 tccggacttc cttaacacag agagatgatg acgaggctct acggagctga agtaactgat 1560 accacacttc caggaaaagc cactaagcga aaggctttac taaaccgtac tgaaaaccga 1620 cacaggtggt caggtagaga atactcaggc gcttgagaga actcgggtga aggaactagg 1680 caaaatagca ccgtaacttc gggagaaggt gcgccggcgt agattgtaag ggctagcccc 1740 40 tgaaggttga accggtcgaa gataccagct ggctgcaact gtttattaaa aacacagcac 1800 tctgcaaaca cgaaagtgga cgtatagggt gtgatgcctg cccggtgctg gaaggttaat 1860 tgatggtgtc atcgaaagag aagcacctga tcgaagcccc agtaaacggc ggccgtaact 1920 ataacggtcc taaggtagcg aaattccttg tcgggtaagt tccgacctgc acgaatggca 1980 taatgatggc caggetgtct ccaccegaga ctcagtgaaa ttgaaategc cgtgaagatg 2040 45 cggtgtaccc gcggctagac ggaaagaccc cgtgaacctt tactatagct tgacactgaa 2100 cattgaattt tgatgtgtag gataggtggg agcctttgaa gcagtgacgc cagtcattgt 2160 ggaggcgacc ttgaaatacc accetttaac gtttgatgtt ctaacgaaga tgacgaaacg 2220 tggtctcgga cagtgtctgg tgggtagttt gactggggcg gtctcctccc aaagcgtaac 2280 ggaggagcac gaaggtttgc taatcacggt cggacatcgt gaggttagtg caatggtata 2340 50 agcaagetta actgegagae agacaagteg agcaggtaeg aaagtaggte atagtgatee 2400 ggtggttctg aatggaaggg ccatcgctca acggataaaa ggtactccgg ggataacagg 2460 ctgataccgc ccaagagttc atatcgacgg cggtgtttgg cacctcgatg tcggctcatc 2520 acatcctggg gctgaagtag gtcccaaggg tatggctgtt cgccatttaa agtggtacgc 2580 gagetgggtt tagaacgteg tgagacagtt eggteeetat etgeegtggg egtaggatga 2640 55 ttgattgggg ctgctcctag tacgagagga ccggagtgga cgcatcactg gtgttccggt 2700 tgtgtcgcca gacgcattgc cgggtagcta aatgcggaag agataagtgc tgaaagcatc 2760

```
taagcacgaa acttgccaag agatgagtca tccctgactt taagtcagta agggttgttg 2820
     taqactacga cgtagatagg ttgggtgtgt aagtgatgtg agtcattgag ctaaccaata 2880
    ctaattgccc gagaggc
5
     <210> 63
     <211> 2865
     <212> DNA
     <213> Bordetella bronchiseptica
10
     <220>
     <221> modified_base
     <222> (622)
     <223> N = A, C, G or T/U
15
     <400> 63
     gatcaagcga ctaagtgcat atggtggatg ccttggcgat cacaggcgga tgaaggacgt 60
     agtagcctgc gaaaagctgc ggggagctgg caaacaagca ttgatccgca gatatccgaa 120
     tggggaaacc cacggcaagc ggtatccctg gctgaataca taggccagtg gaggcgaacc 180
20
     gggtgaactg aaacatctca gtagctcgag gaaaagaaat caaccgagat tccgaaagta 240
     gtggcgagcg aaatcggaag agcctttacg atttagcatt ttgcatagtc gaacggaatg 300
     gaaagtccgg ccgtagcagg tgatagccct gtagacgaat gcagagtgtg gaactaggcg 360
     taagagaagt agggcgggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
     ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
25
     cccqqaaqqa qtqaaataqa tcctqaaacc gtatgcatac aacagtcgga gcctctttat 540
     qqqqtqacqq cqtacctttt qtataatqqq tcaqcqactt acattcaqtq qcaqcttaac 600
     cgaataggga aggcgtcaga anagcagtcc gaatagggcg ttccagtcgc tgggtgtaga 660
     cccgaaacca gatgatctac ccatggccag gttgaaggca cggtaacacg tgctggagga 720
     ccgaacccac tagtgttgaa aaactagggg atgagctgtg gataggggtg aaaggctaaa 780
30
     caaatctgga aatagctggt teteteegaa aactatttag gtagtgeete aagtattaet 840
     gcaggggta gagcactgtt atggctaggg ggtcatggcg acttaccaaa ccatggcaaa 900
     ctccgaatac ctgcaagtac agcttgggag acagacgacc gggtgctaac gtccggactc 960
     aagagggaaa caacccagac cgccagctaa ggtcccgaat tatcgctaag tgggaaacga 1020
     aqtqqqaaqq cataqacaqt caqqaqqttq qcttagaagc agccaccctt taaagaaagc 1080
35
     qtaataqctc actgatcgag tcgtcctgcg cggaagatgt aacggctaag cgataaaccg 1140
     aagctgcggg tgtgcacttt tagtgcagcg gtaggagagc gttctgtaag cctgcgaagg 1200
     tggcttgtaa aggctgctgg aggtatcaga agtgcgaatg ctgacatgag tagccataaa 1260
     gggggtgaaa agccccctcg ccgtaagtcc aaggtttcct gcgcaacgtt catcggcgca 1320
     gggtgagtcg gcccctaagg cgaggcagag atgcgtagct gatgggaagc tggttaatat 1380
40
     tccagcaccg tcgtacagtg cgatgggggg acggatcgcg gaaggtcatc agggtgttgg 1440
     acgtccctgt tgctgcattg aagatggcgc ttaggcaaat ccgggcgcga gaatcaaggg 1500
     tgtggcacga gcgagcaagt ctcgcgaagt gattggaagt ggttccaaga aaagcctcta 1560
     agetteaget gtacgagace gtacegeaaa eegacacagg tgggaeggga tgaatattee 1620
     aaggcgcttg agagaactca ggagaaggaa ctcggcaaat tgataccgta acttcgggag 1680
45
     aaggtatacc ctggtagtgt gaagcctgcg cgctgagcat gaaggggtcg cagagaatcg 1740
     gtggctgcga ctgtttatta aaaacacagc actctgcaaa gacgaaagtc gacgtatagg 1800
     gtgtgacgcc tgcccggtgc cggaaggtta agtgatgggg tgcaagctct tgatcgaagc 1860
     cccggtaaac ggcggccgta actataacgg tcctaaggta gcgaaattcc ttgtcgggta 1920
     agttccgacc tgcacgaatg gcgtaacgat ggccacactg tctcctcctg agactcagcg 1980
50
     aagttgaagt gtttgtgatg atgcaatcta cccgcggcta gacggaaaga ccccatgaac 2040
     ctttactgta gctttgcatt ggactgtgaa ccggcctgtg taggataggt gggaggcgca 2100
     gaactcgagt cgccagattc gagggagcca tccttgaaat accaccctgg tttgtttgcg 2160
     gttctaacct tggtccgtta tccggatcgg ggacagtgca tggtaggcag tttgactggg 2220
     geggteteet eccaaagegt aaeggaggag ttegaaggta egetaggtae ggteggaaat 2280
55
     cgtgctgata gtgcaatggc ataagcgtgc ttgactgtga gactgacagt gaacaggtgc 2340
     gaacgggaca tagtgatccg gtggttctga tggaagggcc atcgctcaac ggataaaggt 2400
```

```
actctgggat aacaggctga taccgcccaa gagttcatat cgacggcggt gtttggcacc 2460
    tegatgtegg etcateteat cetggggetg tageeggtee aagggtatge tgttegeeat 2520
    ttaaagaggt acgtgagctg ggtttagaaa cgtcgtgaga cagtttggtc cctatctgcc 2580
    gtgggcgttg gatacttgaa caggagcctg ctcctagtac gagaggaccg gagtggacgt 2640
    acctctggtg taccggttgt catgccaatg gcattgccgg gtagctaagt acggaagaga 2700
    taaccgctga aggcatctaa gcgggaaact cgtctgaaga ttaggtatcc cggggactag 2760
    atccccctga agggtcgttc gagaccagga cgttgatagg tcgggtgtgg aagcgcagta 2820
    atgcgttaag ctaaccgata ctaattgccc gtgaggctta atcct
10
     <210> 64
     <211> 2865
    <212> DNA
    <213> Bordetella parapertussis
15
    <220>
    <221> modified_base
    <222> (624)
     <223> N = A, C, G or T/U
20
     <400> 64
    gatcaagcga ctaagtgcat atggtggatg ccttggcgat cacaggcgat gaaggacgta 60
    gtagcctgcg aaaagctgcg gggagctggc aaacaagcat tgatccgcag atatccgaat 120
    ggggaaaccc acggcaagcg gtatccctgg ctgaatacat aggccagtgg aggcgaaccg 180
25
    ggtgaactga aacatctcag tagctcgagg aaaagaaatc aaccgagatt ccgaaagtag 240
    tggcgagcga aatcggaaga gcctttacga tttagcattt tgcatagtcg aacggaatgg 300
    aaagtccggc cgtagcaggt gatagccctg tagacgaaat gcagagtgtg gaactaggcg 360
    taagagaagt agggcgggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
    ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
30
    cccggaagga gtgaaataga tcctgaaacc gtatgcatac aaacagtcgg agcctcttta 540
    tggggtgacg gcgtaccttt tgtataatgg gtcagcgact tacattcagt ggcgagctta 600
    accgaatagg gaaggcgtca gaanagcagt ccgaataggg cgtccagtcg ctgggtgtag 660
    acccgaaacc agatgatcta cccatggcca ggttgaaggc acggtaacac gtcgtggagg 720
    accgaaccca ctagtgttga aaaactaggg gatgagctgt ggataggggt gaaaggctaa 780
35
    acaaatctgg aaatagctgg ttctctccga aaactattta ggtagtgcct caagtattac 840
    tgcaggggt agagcactgt tatggctagg gggtcatggc gacttaccaa accatggcaa 900
    actocgaata cotgoaagta cagottggga gacagacgac cgggtgctaa cgtccggact 960
    caagagggaa acaacccaga ccgccagcta aggtcccgaa ttatcgctaa gtgggaaacg 1020
    aagtgggaag gcatagacag tcaggaggtt ggcttagaag cagccaccct ttaaagaaag 1080
40
    cgtaatagct cactgatcga gtcgtcctgc gcggaagatg taacggctaa gcgataaacc 1140
    gaagetgegg gtgtgeactt ttagtgeage ggtaggagag cgttetgtaa geetgegaag 1200
    gtggcttgta aaggctgctg gaggtatcag aagtgcgaat gctgacatga gtagcgataa 1260
    agggggtgaa aagccccctc gccgtaagtc caaggtttcc tgcgcaacgt tcatcggcgc 1320
    agggtgagtc ggcccctaag gcgaggcaga gatgcgtagc tgatgggaag ctggttaata 1380
45
    ttccagcacc gtcgtacagt gcgatggggg gacggatcgc ggaaggtcat cagggtgttg 1440
    gacgtccctg ttgctgcatt gaagatggcg cttaggcaaa tccgggcgcg agaatcaagg 1500
    gtgtggcacg agcgagcaag tetegcgaag tgattggaag tggttccaag aaaagcetet 1560
    aagetteage tgtacgagae egtacegeaa acegacaeag gtgggaeggg atgaatatte 1620
    caaggcgctt gagagaactc aggagaagga actcggcaaa ttgataccgt aacttcggga 1680
50
    gaaggtatac cotggtagtg tgaagcotgc gcgctgagca tgaaggggtc gcagagaatc 1740
    ggtggctgcg actgtttatt aaaaacacag cactctgcaa agacgaaagt cgacgtatag 1800
    ggtgtgacgc ctgcccggtg ccggaaggtt aagtgatggg gtgcaagctc ttgatcgaag 1860
    ccccqqtaaa cggcggccgt aactataacg gtcctaaggt agcgaaattc cttgtcgggt 1920
    aagtteegae etgeaegaat ggegtaaega tggeeacaet gteteeteet gagaeteage 1980
55
    gaagttgaag tgtttgtgat gatgcaatct acccgcggct agacggaaag accccatgaa 2040
    cctttactgt agctttgcat tggactgtga accggcctgt gtaggatagg tgggaggcgc 2100
```

```
agaactcgag tegecagatt cgagggagec atecttgaaa taccaccetg gtttgtttgc 2160
     ggttctaacc ttggtccgtt atccggatcg gggacagtgc atggtaggca gtttgactgg 2220
     ggcggtctcc tcccaaagcg taacggagga gttcgaaggt acgctaggta cggtcggaaa 2280
     tegtgetgat agtgeaatgg cataagegtg ettgactgtg agaetgacag tegaacaggt 2340
     gcgaacggga catagtgatc cggtggttct gatggaaggg ccatcgctca acggataaag 2400
     gtactctggg ataacaggct gataccgccc aagagttcat atcgacggcg gtgtttggca 2460
     cctcgatgtc ggctcatctc atcctggggc tgtagccggt ccaagggtat gctgttcgcc 2520
     atttaaagag gtacgtgagc tgggtttaga aacgtcgtga gacagtttgg tccctatctg 2580
     ccgtgggcgt tggatacttg aacaggagcc tgctcctagt acgagaggac cggagtggac 2640
10
     gtacctctgg tgtaccggtt gtcatgccaa tggcattgcc gggtagctaa gtacggaaga 2700
     gataaccgct gaaggcatct aagcggaaac tcgtctgaag attaggtatc ccgggactag 2760
     atccccctga agggtcgttc gagaccagga cgttgatagg tcgggtgtgg aagcgcagta 2820
     atgcgttaag ctaaccgata ctaattgccc gtgaggcttg atcct
15
     <210> 65
     <211> 2864
     <212> DNA
     <213> Bordetella pertussis
20
     <220>
     <221> modified base
     <222> (624)
     <223> N = A, C, G or T/U
25
     <400> 65
     gatcaagcga ctaagtgcat atggtggatg ccttggcgat cacaggcgat,gaaggacgta 60
     gtagcctgcg aaaagctgcg gggagctggc aaacaagcat tgatccgcag atatccgaat 120
     ggggaaaccc acggcaagcg gtatccctgg ctgaatacat aggccagtgg aggcgaaccg 180
30
     ggtgaactga aacatctcag tagctcgagg aaaagaaatc aaccgagatt ccgaaagtag 240
     tggcgagcga aatcggaaga gcctttacga tttagcattt tgcatagtcg aacggaatgg 300
     aaagtccggc cgtagcaggt gatagccctg tagacgaaat gcagagtgtg gaactaggcg 360
     taagagaagt agggegggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
    ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
35
     cccggaagga gtgaaataga tcctgaaacc gtatgcatac aaacagtcgg agcctcttta 540
     tggggtgacg gcgtaccttt tgtataatgg gtcagcgact tacattcagt ggcgagctta 600
     accgaatagg gaaggcgtca gaanagcagt ccgaataggg cgtccagtcg ctgggtgtag 660
     accegaaacc agatgateta eccatggeca ggttgaagge aeggtaacac gtegtggagg 720
     accgaaccca ctagtgttga aaaactaggg gatgagctgt ggataggggt gaaaggctaa 780
40
     acaaatctgg aaatagctgg ttctctccga aaactattta ggtagtgcct caagtattac 840
     tgcaggggt agagcactgt tatggctagg gggtcatggc gacttaccaa accatggcaa 900
     actocgaata cotgoaagta cagottggga gacagacgao cgggtgotaa cgtcoggact 960
     caagagggaa acaacccaga ccgccagcta aggtcccgaa ttatcgctaa gtgggaaacg 1020
     aagtgggaag gcatagacag tcaggaggtt ggcttagaag cagccaccct ttaaagaaag 1080
45
     cgtaatagct cactgatcga gtcgtcctgc gcggaagatg taacggctaa gcgataaacc 1140
     gaagctgcgg gtgtgcactt ttagtgcagc ggtaggagag cgttctgtaa gcctgcgaag 1200
     gtggcttgta aaggctgctg gaggtatcag aagtgcgaat gctgacatga gtagcgataa 1260
     agggggtgaa aagccccctc gccgtaagtc caaggtttcc tgcgcaacgt tcatcggcgc 1320
     agggtgagtc ggcccctaag gcgaggcaga gatgcgtagc tgatgggaag ctggttaata 1380
50
    ttccagcacc gtcgtacagt gcgatggggg gacggatcgc ggaaggtcat cagggtgttg 1440
    gacgtccctg ttgctgcatt gaagatggcg cttaggcaaa tccgggcgcg agaatcaagg 1500
    gtgtggcacg agcgagcaag tctcgcgaag tgattggaag tggttccaag aaaagcctct 1560
    aagetteage tgtacgagae egtacegeaa acegacacag gtgggaeggg atgaatatte 1620
    caaggcgctt gagagaactc aggagaagga actcggcaaa ttgataccgt aacttcggga 1680
55
    gaaggtatac cctggtagtg tgaagcctgc gcgctgagca tgaaggggtc gcagagaatc 1740
    ggtggctgcg actgtttatt aaaaacacag cactctgcaa agacgaaagt cgacgtatag 1800
```

```
ggtgtgacgc ctgcccggtg ccggaaggtt aagtgatggg gtgcaagctc ttgatcgaag 1860
     ccccggtaaa cggcggccgt aactataacg gtcctaaggt agcgaaattc cttgtcgggt 1920
     aagtteegae etgeaegaat ggegtaaega tggeeaeaet gteteeteet gagaeteage 1980
     gaagttgaag tgtttgtgat gatgcaatct acccgcggct agacggaaag accccatgaa 2040
     cctttactgt agctttgcat tggactgtga accggcctgt gtaggatagg tgggaggcgc 2100
     agaactcgag tcgccagatt cgagggagcc atccttgaaa taccaccctg gtttgtttgc 2160
     ggttctaacc ttggtccgtt atccggatcg gggacagtgc atggtaggca gtttgactgg 2220
     ggcggtctcc tcccaaagcg taacggagga gttcgaaggt acgctaggta cggtcggaaa 2280
     tegtgetgat agtgeaatgg cataagegtg ettgactgtg agactgacag tegaacaggt 2340
10
     gcgaacggga catagtgatc cggtggttct gatggaaggg ccatcgctca acggataaag 2400
     gtactctggg ataacaggct gataccgccc aagagttcat atcgacggcg gtgtttggca 2460
     cctcgatgtc ggctcatctc atcctggggc tgtagccggt ccaagggtat gctgttcgcc 2520
     atttaaagag gtacgtgagc tgggtttaaa acgtcgtgag acagtttggt ccctatctgc 2580
     cgtgggcgtt ggatacttga acaggagcct gctcctagta cgagaggacc ggagtggacg 2640
15
     tacctctggt gtaccggttg tcatgccaat ggcattgccg ggtagctaag tacggaagag 2700
     ataaccgctg aaggcatcta agcggaaact cgtctgaaga ttaggtatcc cgggactaga 2760
     tececetgaa gggtegtteg agaceaggae gttgataggt egggtgtgga agegeagtaa 2820
     tgcgttaagc taaccgatac taattgcccg tgaggcttga tcct
20
     <210> 66
     <211> 2878
     <212> DNA
     <213> Burkholderia cepacia
25
     <400> 66
     ggtcaagcga acaagtgcat gtggtggatg ccttggcgat cacaggcgat gaaggacgcg 60
     gtagcctgcg aaaagctacg gggagctggc aaacaagctt tgatccgtag atgtccgaat 120
     ggggaaaccc actccttttg gagtatccat ggctgaatac ataggccatg cgaaggaacg 180
30
     cggtgaactg aaacatctaa gtaaccgcag gaaaagaaat caaccgagat tcccaaagta 240
     gtggcgagcg aaatgggatg agcettgcac tetttatttg tattgttage egaacgetet 300
     ggaaagtgcg gccatagcag gtgatagccc tgtaggcgaa aacagtatga aagaactagg 360
     tgtgcgacaa gtagggcggg acacgtgaaa tcctgtctga agatgggggg accatcctcc 420
     aaggctaaat actcgtgatc gaccgatagt gaaccagtac cgtgagggaa aggcgaaaag 480
35
     aaccccggga ggggagtgaa atagatcctg aaaccgcatg catacaaaca gtcggagcct 540
     cgtaaggggt gacggcgtac cttttgtata atgggtcagc gacttacgtt cagtagcaag 600
     cttaaccgta tagggcaggc gtaggaaagg agtccgaata gggcgttcag ttgctgggcg 660
     tagacccgaa accaggtgat ctatccatgg ccaggatgaa ggtgcggtaa cacgtactgg 720
     aggtccgaac ccactaacgt tgaaaagtta ggggatgagc tgtggatagg ggtgaaaggc 780
40
     taaacaaacc tggaaatagc tggttctctc cgaaaactat ttaggtagtg cctcgtgtct 840
     cacctteggg ggtagageac tgtcatggtt ggggggtcta ttgcagatta ccccgccata 900
     gcaaactccg aataccgaag agtgcaatca cgggagacag acatcgggtg ctaacgtccg 960
     gtgtcaagag ggaaacaacc cagaccgcca gctaaggtcc ccaaatatag ctaagtggga 1020
     aacgaagtgg gaaggctaaa acagtcagga ggttggctta gaagcagcca ccctttaaag 1080
45
     aaagcgtaat agctcactga tcgagtcgtc ctgcgcggaa gatgtaacgg ggctaagcta 1140
     tataccgaag ctgcggatgc gtgctttgca cgatggtagg agagcgttcc gtaagcctgc 1200
     gaaggtgcct tgtaaagggt gctggaggta tcggaagtgc gaatgctgac atgagtagcg 1260
     ataaaggggg tgaaaggccc cctcgccgta agcccaaggt ttcctacgca acgttcatcg 1320
    gcgtagggtg agtcggcccc taaggcgagg cagaaatgcg tagctgatgg gaagcaggtc 1380
50
    aatatteetg caccattgtt agatgegatg gggggaegga tegeggaagg ttgteegggt 1440
    gttggaagtc ccggtcgctg cattggagaa ggcgcttagg caaatccggg cgcagaattc 1500
    aagggtgtgg cgcgagctcc ttcgggagcg aagcaattgg aagtggttcc aagaaaagcc 1560
    tctaagcttc agtctaacga tgaccgtacc gcaaaccgac acaggtgggc gagatgagta 1620
     ttctaaggcg cttgagagaa ctcgggagaa ggaactcggc aaattggtac cgtaacttcg 1680
55
    ggataaggta cgcccttgta gcttgactgg cctgcgccag gagggtgaag gggttgcaat 1740
     aaactggtgg ctgcgactgt ttaataaaaa cacagcactc tgcaaacacg aaagtggacg 1800
```

```
tatagggtgt gacgcctgcc cggtgccgga agattaaatg atggggtgca agctcttgat 1860
     tgaagtcccg gtaaacggcg gccgtaacta taacggtcct aaggtagcga aattccttgt 1920
     cgggtaagtt ccgacctgca cgaatggcgt aacgatggcc acactgtctc ctcccgagac 1980
     tragraagt tgaagtgttt gtgatgatgr aatrtacreg rggrtagarg gaaagareer 2040
     atgaaccttt actgtagctt tgcattggac tttgaaccga tctgtgtagg ataggtggga 2100
     ggctatgaaa coggaacgct agtttoggtg gagccgtcct tgaaatacca coctggtttg 2160
     tttgaggttc taaccttggc ccgtgatccg ggtcggggac agtgcatggt aggcagtttg 2220
     actggggcgg tctcctccca aagcgtaacg gaggagtacg aaggtacgct aggtacggtc 2280
     ggaaatcgtg ctgatagtgc aatggcataa gcgtgcttaa ctgcgagacc gacaagtcga 2340
10
     gcaggtgcga aagcaggtca tagtgatccg gtggttctgt atggaagggc catcgctcaa 2400
     cggataaaag gtactctggg gataacaggc tgataccgcc caagagttca tatcgacggc 2460
     ggtgtttggc acctcgatgt cggctcatct catcctgggg ctgtagccgg tcccaagggt 2520
     atggctgttc gccatttaaa gaggtacgtg agctgggttt aaaacgtcgt gagacagttt 2580
     ggtccctatc tgccgtgggc gttggatatt tgaagggggc tgctcctagt acgagaggac 2640
15
     cggagtggac gaacctctgg tgtaccggtt gtcacgccag tggcatcgcc gggtagctat 2700
     gttcggaaga gataaccgct gaaagcatct aagcgggaaa ctcgccttaa gatgagatat 2760
     ccctggggac tagatcccct tgaagggtcg ttcgagacca ggacgttgat aggtcaggtg 2820
     tgtaagcgca gtaatgcgtt cagctaactg atactaattg cccgtaaggc ttgatcct
20
     <210> 67
     <211> 2882
     <212> DNA
     <213> Burkholderia mallei
25
     <400> 67
     ggtcaagcga acaagtgcat gtggtggatg ccttggcgat cacaggcgat gaaggacgcg 60
    gtagcctgcg aaaagctacg gggagctggc aaacgagctt tgatccgtag atgtccgaat 120
    ggggaaaccc ggcccttttg ggtcatccta gactgaatac ataggtctag tgaggcgaac 180
30
     gcggtgaact gaaacatcta agtaaccgca ggaaaagaaa tcaaccgaga ttcccaaagt 240
     agtggcgagc gaaatgggaa gagcctgtac tctttatttg tattgttagc cgaacgctct 300
     ggaaagtgcg gccatagcag gtgatagccc tgtaggcgaa aacagtatga aagaactagg 360
     tgtacgacaa gtagggcggg acacgtgaaa teetgtetga agatgggggg accateetee 420
     aaggetaaat actegtgate gacegatagt gaaceagtae egtgagggaa aggegaaaag 480
35
     aaccccggga ggggagtgaa atagatcctg aaaccgcatg catacaaaca gtcggagcct 540
     cttcgggggt gacggcgtac cttttgtata atgggtcagc gacttacgtt cagtagcaag 600
     cttaaccgaa tagggcaggc gtagcgaaag cgagtccgaa tagggcgttc agttgctggg 660
     cgtagacccg aaaccaggtg atctatccat ggccaggatg aaggtgcggt aacacgtact 720
     ggaggtccga acccactaac gttgaaaagt taggggatga gctgtggata ggggtgaaag 780
40
     gctaaacaaa cctggaaata gctggttctc tccgaaaact atttaggtag tgcctcgtgt 840
     ctcaccttcg ggggtagagc actgtcatgg ttggggggtc tattgcagat taccccgcca 900
     tagcaaactc cgaataccga agagtgcaat cacgggagac agacatcggg tgctaacgtc 960
     cggtgtcaag agggaaacaa cccagaccgc cagctaaggt ccccaaatat ggctaagtgg 1020
     gaaacgaagt gggaaggcta aaacagtcag gaggttggct tagaagcagc caccctttaa 1080
45
     agaaagegta atageteaet gategagteg teetgegegg aagatgtaae ggggetaage 1140
     catataccga agctgcggat gcgagctagt ctcgcatggt aggagagcgt tccgtaagcc 1200
     tgcgaaggtg cgttgaaaag cgtgctggag gtatcggaag tgcgaatgct gacatgagta 1260
    gcgataaagg gggtgaaagg ccccctcgcc gtaagcccaa ggtttcctac gcaacgttca 1320
     teggegtagg gtgagtegge eectaaggeg aggeagaaat gegtagetga tgggaageag 1380
50
    gtcaatattc ctgcaccgtc gttagatgcg atggggggac ggatcgcgga aggttgtccg 1440
    ggtgttggaa gtcccggtcg ctgcattgga gaaggcgctt aggcaaatcc gggcgcagga 1500
     ttcaagggtg tggcgcgagc tccttcggga gcgaagcaat tggaagtggt tccaagaaaa 1560
    gcctctaagc ttcagtctaa cgatgaccgt accgcaaacc gacacaggtg ggcgagatga 1620
    gtattctaag gcgcttgaga gaactcggga gaaggaactc ggcaaattgg taccgtaact 1680
55
     tegggataag gtacgeectg gtagettgae tggeetgege cagaagggtg aaggggttge 1740
     aataaactgg tggctgcgac tgtttaataa aaacacagca ctctgcaaac acgaaagtgg 1800
```

PCT/US02/41014

```
acgtataggg tgtgacgcct gcccggtgcc ggaagattaa atgatggggt gcaagctctt 1860
     gattgaagtc coggtaaacg goggcogtaa ctataacggt cotaaggtag cgaaattoot 1920
     tgtcgggtaa gttccgacct gcacgaatgg cgtaacgatg gccacactgt ctcctcccga 1980
     gactcagega agttgaagtg tttgtgatga tgcaatetae eegeggetag aeggaaagae 2040
 5
     cccatgaacc tttactgtag ctttgcattg gactttgaac cgatctgtgt aggataggtg 2100
     ggaggetatg aaaceggaat getagttteg gtggageegt eettgaaata eeaceetggt 2160
     ttgtttgagg ttctaacctt ggcccgtgat ccgggtcggg gacagtgcat ggtaggcagt 2220
     ttgactgggg cggtctcctc ccaaagcgta acggaggagt acgaaggtac gctaggtacg 2280
     gtcggaaatc gtgctgatag tgcaatggca taagcgtgct taactgcgag accgacaagt 2340
10
    cgagcaggtg cgaaagcagg tcatagtgat ccggtggttc tgtatggaag ggccatcgct 2400
     caacqgataa aaggtactet ggggataaca ggetgatace geecaagagt teatategae 2460
     ggcggtgttt ggcacctcga tgtcggctca tctcatcctg gggctgtagc cggtcccaag 2520
     ggtatggctg ttcgccattt aaagaggtac gtgagctggg tttaaaacgt cgtgagacag 2580
     tttggtccct atctgccgtg ggcgttggaa gtttgaaggg ggctgctcct agtacgagag 2640
15
     gaccggagtg gacgaacete tggtgtaccg gttgtgacge cagtcgcate gccgggtage 2700
     tatgttcgga agagataacc gctgaaagca tctaagcggg aaactcgcct taagatgaga 2760
     cttccccggg gacttgatcc ccttgaaggg tcgttcgaga ccaggacgtt gataggtcgg 2820
     gtgtgtaagc gcagtaatgc gttcagctaa ccgatactaa ttgcccgtac ggcttgatcc 2880
                                                                       2882
     ta
20
     <210> 68
     <211> 2882
     <212> DNA
25
     <213> Burkholderia pseudomallei
     <400> 68
     ggtcaagcga acaagtgcat gtggtggatg ccttggcgat cacaggcgat gaaggacgcg 60
    gtagcctgcg aaaagctacg gggagctggc aaacgagctt tgatccgtag atgtccgaat 120
30
     ggggaaaccc ggcccttttg ggtcatccta gactgaatac ataggtctag tgaggcgaac 180
     gcggtgaact gaaacatcta agtaaccgca ggaaaagaaa tcaaccgaga ttcccaaagt 240
     agtggcgagc gaaatgggaa gagcctgtac tctttatttg tattgttagc cgaacgctct 300
     ggaaagtgcg gccatagcag gtgatagccc tgtaggcgaa aacagtatga aagaactagg 360
     tgtacgacaa gtagggcggg acacgtgaaa tcctgtctga agatgggggg accatcctcc 420
35
     aaggctaaat actcgtgatc gaccgatagt gaaccagtac cgtgagggaa aggcgaaaag 480
     aaccccggga ggggagtgaa atagatcctg aaaccgcatg catacaaaca gtcggagcct 540
     cttcgggggt gacggcgtac cttttgtata atgggtcagc gacttacgtt cagtagcaag 600
     cttaaccgaa tagggcaggc gtagcgaaag cgagtccgaa tagggcgttc agttgctggg 660
     cgtagacccg aaaccaggtg atctatccat ggccaggatg aaggtgcggt aacacgtact 720
40
     ggaggtccga acccactaac gttgaaaagt taggggatga gctgtggata ggggtgaaag 780
     gctaaacaaa cctggaaata gctggttctc tccgaaaact atttaggtag tgcctcgtgt 840
     ctcaccttcg ggggtagagc actgtcatgg ttggggggtc tattgcagat taccccgcca 900
     tagcaaactc cgaataccga agagtgcaat cacgggagac agacatcggg tgctaacgtc 960
     cggtgtcaag agggaaacaa cccagaccgc cagctaaggt ccccaaatat ggctaagtgg 1020
45
     gaaacgaagt gggaaggcta aaacagtcag gaggttggct tagaagcagc caccctttaa 1080
     agaaagegta atageteact gategagteg teetgegegg aagatgtaac ggggetaage 1140
     catataccga agctgcggat gcgagctagt ctcgcatggt aggagagcgt tccgtaagcc 1200
     tgcgaaggtg cgttgaaaag cgtgctggag gtatcggaag tgcgaatgct gacatgagta 1260
     gcgataaagg gggtgaaagg ccccctcgcc gtaagcccaa ggtttcctac gcaacgttca 1320
50
    teggegtagg gtgagtegge ceetaaggeg aggeagaaat gegtagetga tgggaageag 1380
    gtcaatatte etgcacegte gttagatgeg atggggggac ggategegga aggttgteeg 1440
    ggtgttggaa gtcccggtcg ctgcattgga gaaggcgctt aggcaaatcc gggcgcagga 1500
    ttcaagggtg tggcgcgagc gctctagggc gcgaagcaat tggaagtggt tccaagaaaa 1560
    gcctctaagc ttcagtctaa cgatgaccgt accgcaaacc gacacaggtg ggcgagatga 1620
55
    gtattctaag gcgcttgaga gaactcggga gaaggaactc ggcaaattgg taccgtaact 1680
     tegggataag gtaegeeetg gtagettgae tggeetgege cagaagggtg aaggggttge 1740
```

```
aataaactgg tggctgcgac tgtttaataa aaacacagca ctctgcaaac acgaaagtgg 1800
     acgtataggg tgtgacgcct gcccggtgcc ggaagattaa atgatggggt gcaagctctt 1860
    gattgaagtc ccggtaaacg gcggccgtaa ctataacggt cctaaggtag cgaaattcct 1920
     tgtcgggtaa gttccgacct gcacgaatgg cgtaacgatg gccacactgt ctcctcccga 1980
    qactcagcga agttgaagtg tttgtgatga tgcaatctac ccgcggctag acggaaagac 2040
    cccatgaacc tttactgtag ctttgcattg gactttgaac cgatctgtgt aggataggtg 2100
    ggaggctatg aaaccggaac gctagtttcg gtggagccgt ccttgaaata ccaccctggt 2160
    ttgtttgagg ttctaacctt ggcccgtgat ccgggtcggg gacagtgcat ggtaggcagt 2220
    ttgactgggg cggtctcctc ccaaagcgta acggaggagt acgaaggtac gctaggtacg 2280
    gtcggaaatc gtgctgatag tgcaatggca taagcgtgct taactgcgag accgacaagt 2340
10
    cgagcaggtg cgaaagcagg tcatagtgat ccggtggttc tgtatggaag ggccatcgct 2400
    caacqqataa aaqqtactct ggggataaca ggctgatacc gcccaagagt tcatatcgac 2460
    ggcggtgttt ggcacctcga tgtcggctca tctcatcctg gggctgtagc cggtcccaag 2520
    ggtatggctg ttcgccattt aaagaggtac gtgagctggg tttaaaacgt cgtgagacag 2580
15
    tttggtccct atctgccgtg ggcgttggaa gtttgaaggg ggctgctcct agtacgagag 2640
    gaccggagtg gacgaacctc tggtgtaccg gttgtgacgc cagtcgcatc gccgggtagc 2700
     tatgttcgga agagataacc gctgaaagca tctaagcggg aaactcgcct taagatgaga 2760
    cttccccggg gacttgatcc ccttgaaggg tcgttcgaga ccaggacgtt gataggtcgg 2820
    gtgtgtaagc gcagtaatgc gttcagctaa ccgatactaa ttgcccgtac ggcttgatcc 2880
20
     <210> 69
     <211> 2890
25
     <212> DNA
     <213> Neisseria gonorrhoeae
     <400> 69
     ggtcaagtga ataagtgcat caggcggatg ccttggcgat gataggcgac gaaggacgtg 60
30
     taagcctgcg aaaagcgcgg gggagctggc aataaagcta tgattccgcg atgtccgaat 120
     ggggaaaccc actgcattct gtgcagtatc ctaagttgaa tacataggct tagagaagcg 180
     aaccoggaga actgaaccat ctaagtacco ggaggaaaag aaatcaaccg agattccgca 240
     agtagtggcg agcgaacgcg gaggagcctg tacgtaataa ctgtcgagat agaagaacaa 300
    gctgggaagc ttgaccatag cgggtgacag tcccgtattc gaaatctcaa cagcggtact 360
35
     aagcgtacga aaagtagggc gggacacgtg aaatcctgtc tgaatatggg gggaccatcc 420
     tccaaggcta aatactcatc atcgaccgat agtgaaccag taccgtgagg gaaaggcgaa 480
     aagaaccccg ggagggaagt gaaacagaac ctgaaacctg atgcatacaa acagtgggag 540
     cgccctagtg gtgtgactgc gtaccttttg tataatgggt caacgactta cattcagtag 600
     cgagcttaac cggatagggg aggcgtaggg aaaccgagtc ttaatagggc gatgagttgc 660
40
     tgggtgtaga cccgaaaccg agtgatctat ccatggtcag gttgaaggtg ccgtaacagg 720
     tactggagga ccgaacccac gcatgttgca aaatgcgggg atgagctgtg ggtaggggtg 780
     aaaggctaaa caaactcgga gatagctggt tctccccgaa aactatttag gtagtgcctc 840
     gagcaagaca ctgatggggg taaagcactg ttatggctag ggggttattg caacttacca 900
     acccatggca aactcagaat accatcaagt ggttcctcgg gagacagaca gcgggtgcta 960
45
     acqtccqttg tcaaqaggga aacaacccag accgccggct aaggtcccaa atgatagatt 1020
     aagtqqtaaa cgaagtggga aggcacagac agccaggatg ttggcttaga agcagccatc 1080
     atttaaagaa agcgtaatag ctcactggtc gagtcgtcct gcgcggaaga tgtaacgggg 1140
     ctcaaatcta taaccgaagc tgcggatgcc ggtttaccgg catggtaggg gagcgttctg 1200
     taggctgatg aaggtgcatt gtaaagtgtg ctggaggtat cagaagtgcg aatgttgaca 1260
50
     tgagtagcga taaagcgggt gaaaagcccg ctcgccgaaa gcccaaggtt tcctacgcaa 1320
     cgttcatcgg cgtagggtaa gtcggcccct aaggcgaggc agaaatgcgt agtcgatggg 1380
     aaacaggtta atatteetgt acttgattea aatgegatgt ggggacggag aaggttaggt 1440
     tggcaagetg ttggaatage ttgtttaage eggtaggtgg aagaettagg caaateeggg 1500
     ttttcttaac accgagaagt gatgacgagt gtctacggac acgaagcaac cgataccacg 1560
55
     cttccaggaa aagccactaa gcttcagttt gaatcgaacc gtaccccaaa ccgacacagg 1620
```

tgggtaggat gagaatteta aggegettga gagaaetegg gagaaggaae teggeaaatt 1680

```
gataccgtaa cttcgggaga aggtatgccc tctaaggtta aggacttgct ccgtaagccc 1740
    cggagggtcg cagagaatag gtggctgcga ctgtttatta aaaacacagc actctgccaa 1800
    cacgaaagtg gacgtatagg gtgtgacgcc tgcccggtgc cggaaggtta attgaagatg 1860
    tgcaagcatc ggatcgaagc cccggtaaac ggcggccgta actataacgg tcctaaggta 1920
    gcgaaattcc ttgtcgggta agttccgacc cgcacgaatg gcgtaacgat ggccacactg 1980
    tctcctcccq agactcagcg aagttgaagt ggttgtgaag atgcaatcta cccgctgcta 2040
    gacggaaaga ccccgtgaac ctttactgta gctttgcatt ggactttgaa gtcacttgtg 2100
    taggataggt gggaggcttg gaagcagaga cgccagtctc tgtggagtcg tccttgaaat 2160
    accaccetgg tgtetttgag gttetaacce agaccegtea teegggtegg ggacegtgca 2220
10
    tggtaggcag tttgactggg gcggtctcct cccaaagcgt aacggaggag ttcgaaggtt 2280
    acctaggtcc ggtcggaaat cggactgata gtgcaatggc aaaaggtagc ttaactgcga 2340
    gaccgacaag tegggeaggt gegaaageag gacatagtga teeggtggtt etgtatggaa 2400
    qqqccatcgc tcaacggata aaaggtactc cggggataac aggctgattc cgcccaagag 2460
    ttcatatcga cggcggagtt tggcacctcg atgtcggctc atcacatcct ggggctgtag 2520
15
    teggteceaa gggtatgget gttegecatt taaagtggta egtgagetgg gtttaaaacg 2580
    tegtgagaca gtttggteee tatetgeagt ggegttggaa gtttgaeggg getgeteeta 2640
    gtacgagagg accggagtgg acgaacctct ggtgtaccgg ttgtaacgcc agttgcatag 2700
    ccgggtagct aagttcggaa gagataagcg ctgaaagcat ctaagcgcga aactcgcctg 2760
    aagatgagac ttcccttgcg gtttaaccgc actaaagggt cgttcgagac caggacgttg 2820
20
    ataggtgggg tgtgggaageg eggtaaegeg tgaagetaae ceataetaat tgeeegtgag 2880
                                                                     2890
    gcttgactct
    <210> 70
25
    <211> 2891
    <212> DNA
    <213> Neisseria meningitidis
    <400> 70
30
    gtcaagtgaa taagtgcatc aggtggatgc cttggcgatg ataggcgacg aaggacgtgt 60
    aagcctgcga aaagcgcggg ggagctggca ataaagcaat gatcccgcga tgtccgaatg 120
    gggaaaccca ctgcattctg tgcagtatcc taagttgaat acatagactt agagaagcga 180
    accoggagaa ctgaaccatc taagtaccog gaggaaaaga aatcaaccga gattccgcaa 240
    gtagtggcga gcgaacgcgg aggagcctgt acgtaataac tgtcgagata gaagaacaag 300
35
    ctgggaaget tgaccatagt gggtgacagt cccgtattcg aaateteaac agcggtacta 360
    agcgtacgaa aagtagggcg gggcacgtga aatcctgtct gaatatgggg ggaccatcct 420
    ccaaggctaa atactcatca tcgaccgata gtgaaccagt accgtgaggg aaaggcgaaa 480
    agaaccccgg gaggggagtg aaacagaacc tgaaacctga tgcatacaaa cagtgggagc 540
    gccctagtgg tgtgactgcg taccttttgt ataatgggtc aacgacttac attcagtagc 600
40
    gagettaacc gaatagggga ggegtaggga aaccgagtet taatagggeg atgagttget 660
    gggtgtagac ccgaaaccga gtgatctatc catggccagg ttgaaggtgc cgtaacaggt 720
    actggaggac cgaacccacg catgttgcaa aatgcgggga tgagctgtgg ataggggtga 780
    aaggctaaac aaactcggag atagctggtt ctccccgaaa actatttagg tagtgcctcg 840
    agcaagacac tgatgggggt aaagcactgt tatggctagg gggttattgc aacttaccaa 900
45
    cccatggcaa actaagaata ccatcaagtg gttcctcggg agacagacag cgggtgctaa 960
    cgtccgttgt caagagggaa acaacccaga ccgccagcta aggtcccaaa tgatagatta 1020
    agtggtaaac gaagtgggaa ggcccagaca gccaggatgt tggcttagaa gcagccatca 1080
    tttaaagaaa gogtaatagc tcactggteg agtcgtcctg cgcggaagat gtaacggggc 1140
    tcaaatctat aaccgaagct gcggatgccg gtttaccggc atggtagggg agcgttctgt 1200
50
    aggctgatga aggtgcattg taaagtgtgc tggaggtatc agaagtgcga atgttgacat 1260
    gagtagcgat aaagcgggtg aaaagcccgc tcgccgaaag cccaaggttt cctgcgcaac 1320
    gttcatcggc gtagggtgag tcggccccta aggcgaggca gaaatgcgta gtcgatggga 1380
    ggcaagctgt tggaatagct tgtttaagcc ggtaggtgga agacttaggc aaatccgggt 1500
55
    cttcttaaca ccgagaagtg acgacgagtg tctacggaca cgaagcaacc gataccacgc 1560
```

ttccaggaaa agccactaag cttcagtttg aatcgaaccg taccgcaaac cgacacaggt 1620

```
gggcaggatg agaattetaa ggcgettgag agaacteagg agaaggaact eggcaaattg 1680
     ataccgtaac ttcgggagaa ggtatgccct ctaaggttaa ggacttgctc cgtaagcccc 1740
    ggagggtcgc agagaatagg tggctgcgac tgtttattaa aaacacagca ctctgctaac 1800
     acgaaagtgg acgtataggg tgtgacgcct gcccggtgct ggaaggttaa ttgaagatgt 1860
    gagagcatcg gatcgaagcc ccagtaaacg gcggccgtaa ctataacggt cctaaggtag 1920
     cgaaattcct tgtcgggtaa gttccgaccc gcacgaatgg cgtaacgatg gccacactgt 1980
     ctcctcctga gactcagcga agttgaagtg gttgtgaaga tgcaatctac ccgctgctag 2040
     acggaaagac cccgtgaacc tttactgtag ctttgcattg gactttgaag tcacttgtgt 2100
    aggataggtg ggaggcttag aagcagagac gccagtctct gtggagccgt ccttgaaata 2160
10
    ccaccetggt gtetttgagg ttetaaccca gaccegteat ccgggtcggg gaccgtgcat 2220
    ggtaggcagt ttgactgggg cggtctcctc ccaaagcgta acggaggagt tcgaaggtta 2280
     cctaggtccg gtcggaaatc ggactgatag tgcaatggca aaaggtagct taactgcgag 2340
     accgacaagt cgagcaggtg cgaaagcagg acatagtgat ccggtggttc tgtatggaag 2400
    ggccatcgct caacggataa aaggtactcc ggggataaca ggctgattcc gcccaagagt 2460
15
    tcatatcgac ggcggagttt ggcacctcga tgtcggctca tcacatcctg gggctgtagt 2520
     cggtcccaag ggtatggctg ttcgccattt aaagtggtac gtgagctggg tttaaaacgt 2580
     cgtgagacag tttggtccct atctgcagtg ggcgttggaa gtttgacggg ggctgctcct 2640
     agtacgagag gaccggagtg gacgaacctc tggtgtaccg gttgtaacgc cagttgcata 2700
    geegggtage taagttegga agagataage getgaaagea tetaagegeg aaactegeet 2760
20
    gaagatgaga cttcccttgc ggtttaaccg cactaaagag tcgttcgaga ccaggacgtt 2820
     gataggtggg gtgtggaagc gcggtaacgc gtgaagctaa cccatactaa ttgctcgtga 2880
    ggcttgactc t
25
     <210> 71
     <211> 2891
     <212> DNA
     <213> Pseudomonas aeruginosa
30
     <400> 71
     ggtcaagtga agaagcgcat acggtggatg ccttggcagt cagaggcgat gaaagacgtg 60
    gtagcctgcg aaaagcttcg gggagtcggc aaacagactt tgatccggag atctctgaat 120
    gggggaaccc acctaggata acctaggtat cttgtactga atccataggt gcaagaggcg 180
     aaccagggga actgaaacat ctaagtaccc tgaggaaaag aaatcaaccg agattccctt 240
35
     agtagtggcg agcgaacggg gattagccct taagcttcat tgattttagc ggaacgctct 300
     ggaaagtgcg gccatagtgg gtgatagccc cgtacgcgaa aggatctttg aagtgaaatc 360
     gagtaggacg gagcacgaga aactttgtct gaacatgggg ggaccatcct ccaaggctaa 420
     atactactga ctgaccgata gtgaaccagt accgtgaggg aaaggcgaaa agaaccccgg 480
     agaggggagt gaaatagaac ctgaaaccgt atgcgtacaa gcagtgggag cctacttgtt 540
40
     aggtgactgc gtaccttttg tataatgggt cagcgactta tattcagtgg caagcttaac 600
     cgtatagggt aggcgtagcg aaagcgagtc ttaatagggc gtttagtcgc tgggtataga 660
     cccgaaaccg ggcgatctat ccatgagcag gttgaaggtt aggtaacact gactggagga 720
     ccgaacccac tcccgttgaa aaggtagggg atgacttgtg gatcggagtg aaaggctaat 780
     caagetegga gatagetggt teteetegaa agetatttag gtagegeete atgtateaet 840
45
     ctgggggta gagcactgtt tcggctaggg ggtcatcccg acttaccaaa ccgatgcaaa 900
     ctccgaatac ccagaagtgc cgagcatggg agacacacgg cgggtgctaa cgtccgtcgt 960
     gaaaagggaa acaacccaga cogccagcta aggtcccaaa gttgtggtta agtggtaaac 1020
     gatgtgggaa ggcttagaca gctaggaggt tggcttagaa gcagccaccc tttaaagaaa 1080
     gcgtaatagc tcactagtcg agtcggcctg cgcggaagat gtaacggggc tcaaaccaca 1140
50
    caccgaaget gegggtgtca cgtaagtgac geggtagagg agegttetgt aageetgtga 1200
     aggtgagttg agaagettge tggaggtate agaagtgega atgetgaeat gagtaaegae 1260
     aatgggtgtg aaaaacaccc acgccgaaag accaagggtt cctgcgcaac gttaatcgac 1320
    gcagggttag tcggttccta aggcgaggct gaaaagcgta gtcgatggga aacaggttaa 1380
    tattcctgta cttctggtta ctgcgatgga gggacggaga aggctaggcc agcttggcgt 1440
55
     tggttgtcca agtttaaggt ggtaggctga aatcttaggt aaatccgggg tttcaaggcc 1500
```

gagagetgat gacgagtegt ettttagatg acgaagtggt tgatgeeatg ettecaagaa 1560

PCT/US02/41014

```
aagettetaa getteaggta accaggaace gtaccecaaa eegacacagg tggtegggta 1620
     gagaatacca aggcgcttga gagaactcgg gtgaaggaac taggcaaaat ggcaccgtaa 1680
     cttcgggaga aggtgcgccg gctagggtga aggatttact ccgtaagctc tggctggtcg 1740
     aagataccag gccgctgcga ctgtttatta aaaacacagc actctgcaaa cacgaaagtg 1800
     gacgtatagg gtgtgacgcc tgcccggtgc cggaaggtta attgatgggg ttagcgcaag 1860
     cgaagetett gategaagee eeggtaaaeg geggeegtaa etataaeggt eetaaggtag 1920
     cgaaatteet tgtegggtaa gtteegaeet geacgaatgg egtaacgatg geggegetgt 1980
     ctccacccga gactcagtga aattgaaatc gctgtgaaga tgcagtgtat ccgcggctag 2040
     acggaaagac cccgtgaacc tttactgtag ctttgcactg gactttgagc ctgcttgtgt 2100
10
     aggataggtg ggaggctttg aagcgtggac gccagttcgc gtggagccat ccttgaaata 2160
     ccaccetgge atgettgagg ttetaactet ggteegtaat eeggategag gacagtgtat 2220
     ggtgggcagt ttgactgggg cggtctcctc ctaaagagta acggaggagt acgaaggtgc 2280
     gctcagaccg gtcggaaatc ggtcgcagag tataaaggca aaagcgcgct tgactgcgag 2340
     acagacacgt cgagcaggta cgaaagtagg tcttagtgat ccggtggttc tgtatggaag 2400
15
     ggccatcgct caacggataa aaggtactcc ggggataaca ggctgatacc gcccaagagt 2460
     tcatatcgac ggcggtgttt ggcacctcga tgtcggctca tcacatcctg gggctgaagc 2520
     cggtcccaag ggtatggctg ttcgccattt aaagtggtac gcgagctggg tttagaacgt 2580
     cgtgagacag ttcggtccct atctgccgtg gacgtttgag atttgagagg ggctgctcct 2640
     agtacgagag gaccggagtg gacgaacctc tggtgttccg gttgtcacgc cagtggcatt 2700
20
     gccgggtagc tatgttcgga aaagataacc gctgaaagca tctaagcggg aaacttgcct 2760
     caagatgaga teteaetggg aacttgatte eeetgaaggg eegtegaaga etaegaegtt 2820
     gataggctgg gtgtgtaagc gttgtgaggc gttgagctaa ccagtactaa ttgcccgtga 2880
     ggcttgacca t
25
     <210> 72
     <211> 2886
     <212> DNA
     <213> Vibrio cholerae
30
     <400> 72
     ggttaagtga ctaagcgtac acggtggatg cctgggcagt cagaggcgat gaaggacgta 60
     ctaacttgcg ataagcgcag ataaggcagt aagagccgtt tgagtctgcg atttccgaat 120
     ggggaaaccc aactgcataa gcagttactg ttaactgaat acataggtta acagagcaaa 180
35
     ccgggggaac tgaaacatct aagtaccccg aggagaagaa atcaaccgag attccggtag 240
     tageggegag egaacetgga ttagecetta ageacteggt gaagtaggtg aacaagetgg 300.
     aaagcttggc gatacagggt gatagccccg taaccgacgc ttcatcgagc gtgaaatcga 360
     gtagggcggg acacgtgata tcctgtctga atatgggggg accatcctcc aaggctaaat 420
     actectgact gacegatagt gaaccagtae egtgaggaaa ggegaaaaga acceetgtga 480
40
     ggggagtgaa atagaacctg aaaccgtgta cgtacaagca gtaggagcac cttcgtggtg 540
     tgactgcgta ccttttgtat aatgggtcag cgacttatat tcagtggcaa ggttaaccgt 600
     ataggggagc cgtagcgaaa gcgagtctta actgggcgct cagtctctgg atatagaccc 660
     gaaaccgggt gatctagcca tgggcaggtt gaaggttgag taacatcaac tggaggaccg 720
     aaccgactaa tgttgaaaaa ttagcggatg acttgtggct aggggtgaaa ggccaatcaa 780
45
     acteggagat agetggttet eccegaaage tatttaggta gegeetegga egaataetae 840
     tgggggtaga gcactgttaa ggctaggggg tcatcccgac ttaccaaccc tttgcaaact 900
     ccgaatacca gtaagtacta tccgggagac acacggcggg tgctaacgtc cgtcgtggag 960
     agggaaacaa cccagaccgc cagctaaggt cccaaagtat tgctaagtgg gaaacgatgt 1020
     gggaaggete agacagetag gatgttgget tagaagcage catcatttaa agaaagegta 1080
50
     atageteact agtegagteg geetgegegg aagatgtaac ggggetaage aatacacega 1140
     agctgcggca atatctttta gatattgggt aggggagcgt tctgtaagcc gttgaaggtg 1200
     aatcgtaagg tttgctggag gtatcagaag tgcgaatgct gacatgagta acgacaaagg 1260
    gggtgaaaaa cctcctcgcc ggaagaccaa gggttcctgt ccaacgttaa tcggggcagg 1320
    gtgagtcgac ccctaaggtg aggccgaaag gcgtaatcga tgggaaacgg gttaatattc 1380
55
    ccgtacttct gactattgcg atggggggac ggagaaggct aggtgggcca ggcgacggtt 1440
     gtcctggttc aagtgcgtag gcttgagagt taggtaaatc cggctctctc taaggctgag 1500
```

```
acacgacgtc gagctactac ggtagtgaag tcattgatgc catgcttcca ggaaaagcct 1560
     ctaagettea gatagteagg aategtaeee caaacegaea caggtggteg ggtagagaat 1620
     accaaggcgc ttgagagaac tcgggtgaag gaactaggca aaatggtacc gtaacttcgg 1680
     gagaaggtac gctcttgatg gtgaagtccc tcgcggatgg agctgacgag agtcgcagat 1740
     accaggtggc tgcaactgtt tattaaaaac acagcactgt gcaaaatcgc aagatgacgt 1800
     atacggtgtg acgcctgccc ggtgccggaa ggttaattga tggggttagc gcaagcgaag 1860
     ctcttgatcg aagccccggt aaacggcggc cgtaactata acggtcctaa ggtagcgaaa 1920
     ttccttgtcg ggtaagttcc gacctgcacg aatggcgtaa tgatggccac gctgtctcca 1980
     cccgagactc agtgaaattg aaatcgctgt gaagatgcag tgtacccgcg gctagacgga 2040
10
     aagaccccgt gaacctttac tacagcttgg cactgaacat tgaacctaca tgtgtaggat 2100
     aggtgggagg ctatgaagac gtgacgccag ttgcgttgga gccgtccttg aaataccacc 2160
     cttgtatgtt tgatgttcta acttagaccc gttatccggg ttgaggacag tgcctggtgg 2220
     gtagtttgac tggggcggtc tcctcccaaa gagtaacgga ggagcacgaa ggtgggctaa 2280
     tcacggttgg acatcgtgag gttagtgcaa tggcataagc ccgcttaact gcgagaatga 2340
15
     cggttcgagc aggtgcgaaa gcaggtcata gtgatccggt ggttctgtat ggaagggcca 2400
     tegeteaacg gataaaaggt acteegggga taacaggetg atacegeeca agagtteata 2460
     tegacggegg tgtttggcac etegatgteg geteateaca teetgggget gaagteggte 2520
     ccaagggtat ggctgttcgc catttaaagt ggtacgcgag ctgggtttag aacgtcgtga 2580
     gacagttcgg tecetatetg ccgtgggcgt tggaagattg aagggggctg etectagtac 2640
20
     gagaggaccg gagtggacga acctetggtg ttcgggttgt gtcgccagac gcattgcccg 2700
     gtagctaagt teggaattga taagegetga aageatetaa gegegaageg ageeetgaga 2760
     tgagtcttcc ctgacagttt aactgtccta aagggttgtt cgagactaga acgttgatag 2820
     geagggtgtg taagegttgt gaggegttga getaacetgt actaattgce egtgaggett 2880
     aaccat
25
     <210> 73
     <211> 2906
     <212> DNA
30
     <213> Yersinia enterocolitica
     <220>
     <221> modified_base
     <222> (1168)..(1178)
35
    <223> N = A, C, G or T/U
     <400> 73
     ggttaagcga ccaagcgtac acggtggatg cctaggcagt cagaggcgat gaaggacgtg 60
     ctaatctgcg aaaagcgtcg gtaaggtgat atgaaccgtt ataaccgacg atacccgaat 120
40
     ggggaaaccc agtgcaattc gttgcactat tgcatggtga atacatagcc atgcaaggcg 180
     aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattccccc 240
     agtagcggcg agcgaacggg gaggagccca gaacctgaat cagcgtatgt gttagtggaa 300
     gcgtctggaa agtcgcacgg tacagggtga tagtcccgta cacaaaaatg catatgttgt 360
     gagttcgatg agtagggcgg gacacgtgac atcctgtctg aatatggggg gaccatcctc 420
45
     caaggctaaa tactcctgac tgaccgatag tgaaccagta ccgtgaggga aaggcgaaaa 480
     gaaccccggc gaggggagtg aaacagaacc tgaaaccgtg tacgtacaag cagtgggagc 540
     accttcgtgg tgtgactgcg taccttttgt ataatgggtc agcgacttat attttgtagc 600
     aaggttaacc gaatagggga gccgtaggga aaccgagtct taactgggcg aatagttgca 660
     aggtatagac ccgaaacccg gtgatctagc catgggcagg ttgaaggttg ggtaacacta 720
50
     actggaggac cgaaccgact aatgttgaaa aattagcgga tgacttgtgg ctgggggtga 780
     aaggccaatc aaaccgggag atagctggtt ctccccgaaa gctatttagg tagcgcctcg 840
     tgaactcatc ttcgggggta gagcactgtt tcggctaggg ggtcatcccg acttaccaaa 900
     ccgatgcaaa ctccgaatac cgaagaatgt tatcacggga gacacacggc gggtgctaac 960
     gtccgtcgtg aagagggaaa caacccagac cgccagctaa ggtcccaaag tcatggttaa 1020
55
     gtgggaaacg atgtgggaag gcacagacag ccaggatgtt ggcttagaag cagccatcat 1080
     ttaaagaaag cgtaatagct cactggtcga gtcggcctgc gcggaagatg taacggggct 1140
```

```
aaaccatgca ccgaagctgc ggcaqcqnnn nnnnnnnnn nnnnnnnngg ggagcgttct 1200
     gtaagccgtt gaaggtgacc tgtgagggtt qctqqaqqta tcagaagtgc gaatgctgac 1260
     ataagtaacg ataatgcggg tgaaaaaccc gcacgccgga agaccaaggg ttcctgtcca 1320
     acgttaatcg gggcagggtg agtcgaccc taaggcgagg ctgaaaggcg tagtcgatqg 1380
     gaaacaggtt aatatteetg tacttggtgt tactgcgaag gggggacgga gaaggctatg 1440
     ctagccgggc gacggttgtc ccggtttaag catgtaggcg gagtgaccag gtaaatccgg 1500
     ttgcttatca acgctgaggt gtgatgacga gtcactacgg tgatgaagta gttgatgcca 1560
     tgcttccagg aaaagcctct aagcatcagg taacatgaaa tcgtacccca aaccgacaca 1620
     ggtggtcagg tagagaatac tcaggcgctt gagagaactc gggtgaagga actaggcaaa 1680
     atggtgccgt aacttcggga gaaggcacgc tgacacgtag gtgaagcggt ttacccgtgg 1740
     agctgaagtc agtcgaagat accagctggc tgcaactgtt tattaaaaac acagcactgt 1800
     gcaaacacga aagtggacgt atacggtgtg acgcctgccc ggtgctggaa ggttaattga 1860
     tggggtcagc gcaagcgaag ctcttgatcg aagccccggt aaacggcggc cgtaactata 1920
     acggtcctaa ggtagcgaaa ttccttgtcg ggtaagttcc gacctgcacg aatggcgtaa 1980
15
     tgatggccag gctgtctcca cccgagactc agtgaaattg aactcgctgt gaagatgcag 2040
     tgtacccgcg gcaagacgga aagaccccgt gaacctttac tatagcttga cactgaacat 2100
     tgagccttga tgtgtaggat aggtgggagg catagaagtg tggacgccag tctgcatgga 2160
     gccaaccttg aaataccacc ctttaatgtt tgatgttcta acteggcccc gtaatccggg 2220
     gtgaggacag tgtcaggtgg gtagtttgac tggggcggtc tcctcccaaa gagtaacgga 2280
     ggagcacgaa ggttagctaa tcacggtcgg acatcgtgag gttagtgcaa aggcataagc 2340
     tagetteact gegagagtga eggetegage aggtaegaaa gtaggtetta gtgateeggt 2400
     ggttctgaat ggaagggcca tcgctcaacg gataaaaggt actccgggga taacaggctg 2460
     ataccgccca agagttcata tcgacggcgg tgtttggcac ctcgatgtcg gctcatcaca 2520
     tectgggget gaagtaggte ceaagggtat ggetgttege catttaaagt ggtacgegag 2580
25
     ctgggtttag aacgtcgtga gacagttcgg tccctatctg ccgtgggcgy tggarraytg 2640
     agrggggctg ctcctagtac gagaggaccg gagtggacgm atcactggtg ttcqqqttqt 2700
     catqccaatq qcaytqcccq qtaqctaaat kcqqaaqaqa taasyqctqa aaqcatctaa 2760
     gcrsqaaact tgccycgaga tgagttctcc ctgagactac aagtctcctg aaggaacqtt 2820
     gaagacgacg acgttgatag gcygggtgtg taagcgcgag ttggcgttga gctaaccggt 2880
30
     actaatgaac cgtgaggctt aacctt
     <210> 74
     <211> 23
35
     <212> DNA
     <213> Artificial Sequence
     <220>
     <223> Description of Artificial Sequence: Synthetic
40
          Primer
     <400> 74
     gggttgcgct cgttacggga ctt
                                                                       23
45
     <210> 75
     <211> 23
     <212> DNA
     <213> Artificial Sequence
50
     <223> Description of Artificial Sequence: Synthetic
          Primer
55
     <400> 75
    gggttgcgct cgttgccgga ctt
                                                                       23
```

5	<210> 76 <211> 23 <212> DNA <213> Artificial Sequence	
10	<220> <223> Description of Artificial Sequence: Synthetic Primer	
	<400> 76 tccccactgc tgcctcccgt agg	23
15		
	<210> 77	
	<211> 23	
	<212> DNA	
20	<213> Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Synthetic Primer	
25	<400> 77	
	caacatetea egacaegage tga	23
30	<210> 78 <211> 23 <212> DNA <213> Artificial Sequence	
35	<220> <223> Description of Artificial Sequence: Synthetic Primer	
	<400> 78	
	tccccactgc tgcctcccgt agg	23
40		
	010 70	
	<210> 79 <211> 22	
	<211> 22 <212> DNA	
45	<213> Artificial Sequence	
	.220.	
	<pre><220> <223> Description of Artificial Sequence: Synthetic</pre>	
	Primer	
50		
	<400> 79	
	ttaccgegge tgetggeacg ga	22
55	<210> 80	
	<211> 23	

	<212> DNA <213> Artificial Sequence	
5	<220> <223> Description of Artificial Sequence: Syprimer	nthetic
10	<400> 80 ccccgtcaat tcctttgagt ttc	23
16	<210> 81 <211> 23 <212> DNA	
15	<213> Artificial Sequence <220>	
	<pre><220> <223> Description of Artificial Sequence: Sy Primer</pre>	rnthetic
20	<400> 81 caacatctca cgacacgagc tga	23
25	<210> 82 <211> 23 <212> DNA <213> Artificial Sequence	
30	<pre><220> <223> Description of Artificial Sequence: Sy Primer</pre>	nthetic
35	<400> 82 tttcaccttt ccctcacggt act	23
40	<210> 83 <211> 23 <212> DNA <213> Artificial Sequence	·
45	<220> <223> Description of Artificial Sequence: Sy Primer	nthetic
	<400> 83 ggttcttttc acctttccct cgc	23
50		
	<210> 84 <211> 23 <212> DNA <213> Artificial Sequence	
55	<220>	

	<pre><223> Description of Artificial Sequence Primer</pre>	: Synthetic	
	<400> 84		
5	tggtttcagg ttctatttca ctc		23
	<210> 85 <211> 22		
10	<212> DNA		
	<213> Artificial Sequence		
	<220>	a - 111 - 112 - 1	
15	<223> Description of Artificial Sequence Primer	: Synthetic	
	<400> 85		
	tttaaccgac aaggaatttc gc		22
20			
	<210> 86		
	<211> 23		
	<212> DNA <213> Artificial Sequence		
25	V2137 Altificial bequence		
	<220>		
	<223> Description of Artificial Sequence Primer	: Synthetic	
30	<400> 86		•
	ggttetttte acettteeet ege		23
25	<210> 87		
35	<211> 15 <212> DNA		
	<212> DNA <213> Artificial Sequence		
	and the state of t		
40	<220>		
40	<223> Description of Artificial Sequence Primer	: Synthetic	
	<400> 87		
	taacctggtc gtaac		15
45			
	<210> 88		
	<211> 14		
	<212> DNA		
50	<213> Artificial Sequence		
	<220>		
	<223> Description of Artificial Sequence	: Synthetic	
55	Primer		
))	<400> 88		

	cccccccc ccc	14
	<210> 89	
5	<211> 16	
-	<212> DNA	
	<213> Artificial Sequence	
	<220>	
10	<223> Description of Artificial Sequence: Synthetic Primer	
	<400> 89	
15	gecectaace tegteg	16
	<210> 90	
	<211> 26	
	<212> DNA	
20	<213> Artificial Sequence	
	<220>	
	<pre><223> Description of Artificial Sequence: Synthetic Primer</pre>	
25		
	<400> 90	
	eggeeetage egggtegtae eteegg	26
30	<210> 91	
-	<211> 26	
	<212> DNA	
	<213> Artificial Sequence	
35	<220>	
	<pre><223> Description of Artificial Sequence: Synthetic Primer</pre>	
	<400> 91	
40	cggccctaac ctggtcgtaa ctccgg	26
	<210> 92	
	<211> 23	
45	<212> DNA	
-	<213> Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Synthetic	
50	Primer	
	<400> 92	23
	aggettegat ceegggatee geg	43